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Association between *Faecalibacterium prausnitzii* and dietary fibre in colonic fermentation in healthy human subjects

Robin F. J. Benus[1,2]*, Tjip S. van der Werf[2], Gjalt W. Welling[1], Patricia A. Judd[3], Moira A. Taylor[4], Hermie J. M. Harmsen[1] and Kevin Whelan[5]

[1]*Departments of Medical Microbiology, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands*
[2]*Internal Medicine, and Pulmonary Diseases and Tuberculosis, Infectious Diseases & Tuberculosis Service, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands*
[3]*Lancashire School of Health and Postgraduate Medicine, University of Central Lancashire, Preston, UK*
[4]*School of Biomedical Sciences, University of Nottingham, Nottingham, UK*
[5]*Nutritional Sciences Division, King's College London, London, UK*

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The intestinal microbiota are a complex ecosystem influencing the immunoregulation of the human host, providing protection from colonising pathogens and producing SCFA as the main energy source of colonocytes. Our objective was to investigate the effect of dietary fibre exclusion and supplementation on the intestinal microbiota and SCFA concentrations. Faecal samples were obtained from healthy volunteers before and after two 14 d periods of consuming formulated diets devoid or supplemented with fibre (14 g/l). The faecal microbiota were analysed using fluorescent *in situ* hybridisation and SCFA were measured using GLC. There were large and statistically significant reductions in the numbers of the *Faecalibacterium prausnitzii* ($P \leq 0{\cdot}01$) and *Roseburia* spp. ($P \leq 0{\cdot}01$) groups during both the fibre-free and fibre-supplemented diets. Significant and strong positive correlations between the proportion of *F. prausnitzii* and the proportion of butyrate during both baseline normal diets were found (pre-fibre free r 0·881, $P=0{\cdot}001$; pre-fibre supplemented r 0·844, $P=0{\cdot}002$). A significant correlation was also found between the proportional reduction in *F. prausnitzii* and the proportional reduction in faecal butyrate during both the fibre-free (r 0·806; $P=0{\cdot}005$) and the fibre-supplemented diet (r 0·749; $P=0{\cdot}013$). These findings may contribute to the understanding of the association between fibre, microbiota and fermentation in health, during enteral nutrition and in disease states such as Crohn's disease.

Dietary fibre: Bacteria: Anaerobic bacteria: Molecular diagnostic techniques: Microbiota: Fermentation

The intestinal microbiota are a complex ecosystem of several hundred microbial species[1]. The microbiota have numerous functions in the maintenance of health, including the ability to decrease colonisation and infection with pathogens, a mechanism referred to as colonisation resistance[2]. The intestinal microbiota also produce SCFA through metabolism of fermentable substrates such as dietary fibre. SCFA stimulate colonic water absorption[3] and, in particular butyrate, are the primary energy source for the colonocytes that constitute the epithelial lining of the large intestine[4]. Butyrate promotes the growth of colonocytes in animal models, preventing mucosal atrophy, and appears to lower the risk of malignant transformation in the colon[5]. Therefore, butyrate in the intestine is beneficial for the integrity of the mucosal barrier function of the colon. However, not all species of the intestinal microbiota produce butyrate, with the predominant butyrate producers belonging to the genus *Faecalibacterium prausnitzii* [6] and *Roseburia* spp.[4].

Colonisation resistance and SCFA production are therefore important functions of the intestinal microbiota[7]. However, when the intestinal microbiota are altered, for example through the use of antimicrobials[8] or by a reduction in fermentable biomass[9], their capacity to exert these important functions is reduced. The major source of fermentable biomass is NSP (i.e. dietary fibre). Both the US Institute of Medicine[10] and the American Dietetic Association[11] have recommended increased dietary fibre consumption as evidence accumulates that higher intakes are related to a lower risk of CVD, and potentially colon cancer, diabetes and obesity. These beneficial effects of dietary fibre may be mediated in part by the augmentation of colonic fermentation and SCFA production.

Research on the impact of dietary fibre on the intestinal microbiota and SCFA production in human subjects is impeded by the ubiquitous presence of fibre in the normal diet, making it difficult to achieve total dietary exclusion.

Abbreviation: FISH, fluorescent *in situ* hybridisation.

* **Corresponding author:** Dr Robin F. J. Benus, fax +31 503619105, email r.f.j.benus@mmb.umcg.nl

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However, one approach to resolving this is through the use of liquid enteral formulas that are used to provide artificial nutrition support. To date, many of the most commonly used standard formulas have been completely devoid of fibre, but formulas supplemented with various types of dietary fibre are now available.

Use of such formulas provides a way of investigating the complete removal of dietary fibre from the diet as well as the effect of addition of specific types and amounts of dietary fibre on the intestinal microbiota and SCFA in healthy human subjects. In addition, they may also enable investigation of two clinically relevant situations. First, patients receiving enteral nutrition in the intensive care unit, and other settings, can experience impairments in gastrointestinal function, including diarrhoea[12]. Second, enteral formula nutrition is effective in inducing remission in active Crohn's disease[13]. Alterations of the intestinal microbiota implicated in disease activity can also be effective in inducing remission[14]. Furthermore, faecal concentrations of *F. prausnitzii* are lower in patients with active inflammatory bowel disease[15], whilst butyrate may be a potential treatment for Crohn's disease[16] through its immunoregulatory effects[17].

Our hypothesis is that the exclusion of dietary fibre will cause a significant change in the numbers of the butyrate producers *F. prausnitzii* and *Roseburia* spp., and thereby reduce SCFA concentrations; in contrast, the addition of dietary fibres will prevent such effects on the intestinal microbiota and SCFA.

We have previously described the effects of consumption of enteral formulas devoid of fibre on the total microbiota and SCFA. The reduction of both total bacterial count and SCFA production that occurred was partially prevented by the addition of fibre and fructo-oligosaccharides to the diet[18]. Interestingly, butyrate concentrations were reduced following both fibre-free and fibre-supplemented formulas. However, in that study there was no extensive analysis of bacterial groups, including the butyrate producers *F. prausnitzii* and *Roseburia* spp., nor was there analysis of the association between specific bacterial groups and the concentrations of SCFA.

The majority of bacteria in the colon are anaerobic, many are hard to culture and enumerating using selective media typically underestimates the numbers of bacteria in faecal samples compared with molecular techniques such as fluorescent *in situ* hybridisation (FISH)[19]. Our extended probe set covers approximately 88 % of the total intestinal microbiota in healthy volunteers, including the butyrate producers *F. prausnitzii* and *Roseburia* spp.[20].

Here we describe an independent blinded reanalysis of the faecal samples from the previous study of liquid formulas devoid of, and supplemented with, fibre[18], using the extended probe set, in order to provide further insights into the relationships between fibre, the intestinal microbiota and faecal SCFA concentrations.

Methods and materials

Subjects

Samples were obtained from healthy volunteers recruited to a prospective, double-blinded, randomised, cross-over trial[18]. The subjects were free of gastrointestinal diseases, or self-reported eating disorders; they were not following a special diet, had not used antibiotics in the previous 3 months and had not consumed products containing prebiotics or probiotics in the previous 1 month. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and was approved by the King's College London Research Ethics Committee (reference 01-62). Written informed consent was obtained from each subject before recruitment.

Protocol

Subjects consumed their normal diet for 14 d (baseline period), then an enteral formula for 14 d (diet period), followed by their normal diet for 6 weeks as a washout phase (baseline period) and then an alternative enteral formula again for 14 d (diet period). All consumption of prebiotics and probiotics was avoided for the duration of the study. During the liquid diet periods, the formulas were the only source of nutrition except for water *ad libitum* and black tea and/or coffee to a maximum of 600 ml/d. Subjects were advised on the amounts of formula required to achieve their calculated total energy expenditure and provided with sufficient formula to maintain their weight. During the diet periods, subjects consumed either a fibre-free enteral formula or a formula supplemented with dietary fibre (14 g/l). The dietary fibre formula consisted of pea fibre (8·9 g/l) and fructo-oligosaccharides (5·1 g/l) and therefore contained 48 % soluble and 52 % insoluble fibre fractions. The nutritional values of both formulas were almost identical except for the difference in fibre content. The order of consumption of each formula was randomised and this randomisation was blinded for both study subjects and researchers. This blinding was maintained for the present analysis.

Dietary intake during the normal diet (baseline) was recorded in a 7 d semi-weighed food diary. The data were entered into a dietary analysis package (CompEat v4; Nutrition Systems, Banbury, Oxon, UK). Intake of the formulas during the fibre-free diet and fibre-supplemented diet was recorded using self-reported diaries[18]. Faecal frequency was calculated from self-reported diaries during the last 7 d of both normal diets periods (baseline), the fibre-free diet and fibre-supplemented diet. Mean daily faecal weight was calculated from a 3 d total faecal collection during the last 3 d of each diet period.

The final faecal specimen from each period was collected and stored for analysis. A sample was frozen at −80°C for analysis of SCFA using GLC, as published earlier[18]. A sample was also processed and stored for analysis of microbiota using FISH[21]. Briefly, samples were diluted 1:10 (w/v) in PBS (NaCl (8 g/l), KCl (0·2 g/l), $Na_2HPO4{\cdot}2H_2O$ (1·44 g/l), KH_2PO_4 (0·24 g/l), pH 7·4) and fixed in 4 % paraformaldehyde in PBS for at least 4 h. Washed cells were re-suspended in PBS–ethanol solution (1:1, v/v) and stored at −20°C before the present analysis.

Fluorescent in situ *hybridisation*

For quantification of the bacteria in these faecal samples, multiple slides with 1 cm^2 wells were prepared for cell counting. Per well, 10 μl of diluted sample were spread. After drying, the cells were fixed to the glass surface with 96 % ethanol

for 10 min. In the present study hybridisation was performed with an extended set of 16S rRNA-targeted probes (Table 1). The probe set used for bacterial groups covers approximately 88 % of the total number of bacteria which hybridise with the EUB338 probe in healthy volunteers[20]. The probes were manufactured by Eurogentec (Seraing, Belgium) and were labelled at the 5′ end with either fluorescein isothiocyanate (FITC) or Cy3.

The samples were hybridised overnight at 50°C in hybridisation buffer (0·9 M-NaCl, 20 mM-2-amino-2-hydroxymethyl-propane-1,3-diol (Tris)-HCl (pH 7·2), 0·1 % SDS (w/v)) containing 9 ng labelled probe per slide. The slides were washed for 20 min in wash buffer (0·9 M-NaCl, 20 mM-Tris-HCl (pH 7·2)), rinsed briefly in Milli-Q and dried using compressed air. Total cells were enumerated after staining with 4′,6-diamidino-2-phenylindole (DAPI). Slides were mounted in Vectashield® (Vector Labs, Burlingame, CA, USA) to minimise fading of the fluorescent signal.

The fluorescent cells in the samples were counted automatically with a Leica® DMRA2 epifluorescence microscope using a modified version of the Leica® Q-win® software (Leica, Wetzlar, Germany)[22]. The detection limit used with this method was set at 10^6 cells/g.

Concentrations are presented per g dry faeces in order to standardise comparisons between samples with different water content. Dry faecal weight was calculated following lyophilisation of duplicate faecal samples.

Statistics

All data were analysed on SPSS for Windows (version 15.0; SPSS, Inc., Chicago, IL, USA). The concentrations of faecal bacteria were log transformed in order to have a normal distribution, and the geometric mean calculated. The log-transformed concentrations and proportions of bacteria were compared between baseline and diet periods using a paired *t* test. To investigate associations between the microbiota and SCFA, the correlation between the proportion of each bacterial group (as percentage of the total cell count) and the proportion of each major SCFA (as percentage of the total) was calculated using a Pearson's correlation coefficient at each time point. To investigate the impact of excluding and supplementing fibre on the associations between the microbiota and SCFA, the change in the proportion of each bacterial group and the change in the proportion of each major SCFA between baseline and diet period was calculated. Then, the correlation between the change in the proportion of each of these was calculated using a Pearson's correlation coefficient. $P<0·05$ was considered statistically significant.

Results

Fourteen healthy subjects, five men and nine women, aged between 21 and 34 years old were included in the original study[18]. Two women withdrew because they were unable to consume the formula as their sole source of nutrition; one woman withdrew because of personal reasons unrelated to the study. One man was excluded after testing positive for *Giardia lamblia* during the study. In total, ten healthy subjects (six women and four men) completed the study and were included in this analysis.

Intakes of fibre (19·6 and 18·0 g/d) and NSP (14·3 and 12·8 g/d) during normal (baseline) diets are similar to values previously recorded in the UK (Table 2).

There was a reduction in faecal frequency between the normal diet and the fibre-free diet (1·0 (SD 0·3) *v.* 0·6 (SD 0·2); $P=0·001$), whilst the reduction between the normal diet and the fibre-supplemented diet (1·1 (SD 0·3) *v.* 0·9 (SD 0·3); $P=0·056$) did not reach statistical significance. Faecal frequency was higher during the fibre-supplemented diet than the fibre-free diet ($P=0·019$). There was a reduction in daily faecal weight between the normal diet and the fibre-free diet (132·4 (SD 68·5) *v.* 43·8 (SD 30·1) g; $P=0·005$) and the normal diet and the fibre-supplemented diet (127·5 (SD 71·2) *v.* 73·2 (SD 37·5) g; $P=0·034$). However, there were no differences in daily faecal weight between the fibre-free and fibre-supplemented diets ($P=0·149$).

In general, the results show a declining trend in all bacterial species during both diet periods, except for bifidobacteria, which increased during the fibre-supplemented diet (Table 3). Interestingly, statistically significant and large reductions in both the concentration and the proportion of the *F. prausnitzii* group and *Roseburia intestinalis* group occurred during both diet periods, irrespective of fibre content, whereas the *Bacteroides* group underwent significant decline in concentration and proportion only during the fibre-free diet period only.

There were significant reductions in concentrations of total SCFA and each of the major SCFA (acetate, propionate, butyrate) following the fibre-free diet, whilst only butyrate

Table 1. Probes used for the detection of the intestinal microbiota

Target	Probe	Label	Sequence	Reference
All bacteria	EUB338	FITC	5′GCTGCCTCCCGTAGGAGT	Amann *et al.* (1990)[35]
Bacteroides/Prevotella	Bac303	FITC	5′CCAATGTGGGGGACCTT	Manz *et al.* (1996)[36]
Eubacterium rectale/Blautia coccoides	Erec482	FITC	5′GCTTCTTAGTCA(G/A)GTACCG	Franks *et al.* (1998)[21]
Roseburia cluster	Rint623	FITC	5′TTCCAATGCAGTACCGGG	Hold *et al.* (2003)[37]
	Rinthelper		5′GTTGAGCCCCGGGCTTT	Aminov *et al.* (2006)[26]
Faecalibacterium prausnitzii group	Fprau645	Cy3	5′CCTCTGCACTACTCAAGAAAAAC	Suau *et al.* (2001)[38]
Atopobium group	Ato291	FITC	5′GGTCGGTCTCTCAACCC	Harmsen *et al.* (2000)[39]
Bifidobacteria	Bif164	FITC	5′CATCCGGCATTACCACCC	Langendijk *et al.* (1995)[40]
Ruminococci	Rbro730	Cy3	5′TAAAGCCCAG(T/C)AGGCCGC	Harmsen *et al.* (2002)[20]
	Rfla729	Cy3	5′AAAGCCCAGTAAGCCGCC	
Enterobacteriaceae	EC1531	Cy3	5′CACCGTAGTGCCTCGTCATCA	Poulsen *et al.* (1994)[41]

FITC, fluorescein isothiocyanate.

Table 2. Nutrient intake during the consumption of normal diet (baseline), fibre-free diet and fibre-supplemented diet*
(Mean values and standard deviations)

	Normal diet (baseline)		Fibre-free diet		Normal diet (baseline)		Fibre-supplemented diet	
Nutrient	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy (kJ/d)	9540	2146	8437	1365	9193	2093	7772	1229
Protein (g/d)	84·3	27·7	80·7	13·0	77·8	21·2	74·5	11·8
Fat (g/d)	87·6	27·4	76·6	12·4	85·0	34·3	70·1	11·2
Carbohydrate (g/d)	271·0	53·9	254·7	41·2	245·9	49·5	235·0	37·2
Fibre (g/d)								
Total fibre	19·6	5·3	0·0		18·0	7·7	27·9	4·4
NSP (excluding FOS)	14·3	4·2	0·0		12·8	5·2	†	
FOS	†		0·0		†		9·3	1·5
Soluble component	†		0·0		†		13·4	2·1
Insoluble component	†		0·0		†		14·5	2·3

FOS, fructo-oligosaccharides.
* Values for the energy, macronutrient and fibre content of the fibre-free diet (Nutren 1·0) and the fibre supplemented diet (Nutren fibre) were provided by Nestlé UK.
† Data not available.

was reduced following the fibre-supplemented diet. There was an increase in the proportion of acetate and a reduction in the proportion of butyrate following both the fibre-free diet and the fibre-supplemented diet. Details of these results are presented elsewhere[18].

There were significant and strong positive correlations between the proportion of *F. prausnitzii* and the proportion of butyrate during the normal diet (Table 4) and these occurred before both the fibre-free (r 0·881; P=0·001) and the fibre-supplemented diet (r 0·844; P=0·002). Interestingly, these correlations were no longer found during either the fibre-free (r 0·359; P=0·308) or the fibre-supplemented diet (r 0·090; P=0·805).

The dynamic effects of switching from a normal diet to a fibre-free or a fibre-supplemented diet were also investigated (Table 5). There was a significant correlation between the change in proportion of *F. prausnitzii* and the change in proportion of butyrate following the fibre-free diet (r 0·806; P=0·005) and between the change in proportion of acetate (r 0·671; P=0·034) and butyrate (r 0·749; P=0·013) following the fibre-supplemented diet. There were no other significant correlations in these changes for any bacterial group and any SCFA.

Discussion

This is the first study to demonstrate a strong correlation between *F. prausnitzii* and butyrate production in healthy volunteers during a normal diet, and that the reduction in *F. prausnitzii* when switching to a fibre-free or

Table 3. Intestinal microbiota during the consumption of the fibre-free and fibre-supplemented diet
(Mean values and standard deviations)

	Normal diet (baseline)		Fibre-free diet period			Normal diet (baseline)		Fibre-supplemented diet period			
	Mean	SD	Mean	SD	P*	Mean	SD	Mean	SD	P*	P†
Bacteria concentration ($\log_{10}$ cells/g dry faeces)											
Total cell count	11·1	0·2	10·9	0·2	0·07	11·1	0·2	10·8	0·4	0·11	0·56
Total bacteria	11·0	0·1	10·8	0·2	0·07	11·0	0·3	10·9	0·3	0·45	0·50
Bacteroides	10·5	0·2	9·7	0·8	0·02	10·4	0·6	9·9	0·9	0·21	0·54
Eubacterium rectale group	10·3	0·2	9·8	0·7	0·12	10·3	0·4	9·8	0·7	0·08	0·94
Roseburia group	9·2	0·3	8·1	0·9	0·01	9·2	0·3	8·2	0·6	<0·01	0·74
Faecalibacterium prausnitzii group	10·1	0·4	8·9	0·7	<0·01	10·1	0·4	9·2	0·7	0·01	0·23
Atopobium group	9·3	0·8	9·3	0·7	0·98	9·2	0·7	9·2	0·9	0·96	0·35
Bifidobacteria	9·2	0·9	9·1	1·3	0·52	9·4	0·6	9·8	0·9	0·15	0·04
Ruminococci	9·9	0·3	9·2	0·8	0·05	9·7	0·8	9·4	0·5	0·15	0·34
Enterobacteriaceae	7·7	0·7	7·3	0·9	0·04	7·4	0·6	7·1	0·4	0·14	0·48
Bacteria proportion (% of total cell count)											
Bacteroides	31·2	20·8	17·5	19·9	0·02	29·8	25·7	24·7	19·0	0·58	0·31
Eubacterium rectale group	20·2	14·0	19·9	22·8	0·97	26·1	37·5	12·1	7·6	0·28	0·26
Roseburia group	1·6	0·9	0·5	0·6	0·02	1·9	1·7	0·6	0·8	0·03	0·84
Faecalibacterium prausnitzii group	17·1	22·7	2·5	4·2	0·04	14·0	13·6	3·3	3·1	0·02	0·48
Atopobium group	3·7	4·2	5·9	5·8	0·35	2·4	2·4	8·8	11·0	0·05	0·26
Bifidobacteria	4·7	5·5	10·6	21·2	0·29	4·6	4·1	20·1	18·3	0·01	0·07
Ruminococci	7·1	3·2	5·9	7·9	0·67	8·2	7·9	6·6	7·3	0·63	0·80
Enterobacteriaceae	0·1	0·1	0·1	0·2	0·42	0·0	0·0	0·0	0·1	0·57	0·19

* P value for diet *v.* baseline.
† P value for fibre-free diet *v.* fibre-supplemented diet.

Table 4. Correlations between the proportion of intestinal microbiota (as percentage of total cells) and the proportion of acetate, propionate and butyrate (as percentage of total SCFA) at the end of each dietary period

	Acetate		Propionate		Butyrate	
	r	*P*	*r*	*P*	*r*	*P*
Bacteroides						
Normal diet (baseline)	−0·470	0·170	0·443	0·200	0·202	0·575
Fibre-free diet	−0·548	0·101	0·910	<0·001*	0·401	0·251
Normal diet (baseline)	−0·208	0·563	0·118	0·746	−0·141	0·698
Fibre-supplemented diet	0·008	0·983	0·365	0·300	0·043	0·907
Eubacterium rectale group						
Normal diet (baseline)	−0·513	0·129	−0·138	0·703	0·950	<0·001*
Fibre-free diet	−0·321	0·365	0·367	0·297	0·171	0·636
Normal diet (baseline)	0·192	0·596	−0·313	0·379	0·502	0·139
Fibre-supplemented diet	0·004	0·992	0·469	0·172	0·076	0·835
Faecalibacterium prausnitzii group						
Normal diet (baseline)	−0·498	0·143	0·021	0·954	0·881	0·001*
Fibre-free diet	−0·365	0·299	0·351	0·319	0·359	0·308
Normal diet (baseline)	0·393	0·261	−0·510	0·132	0·844	0·002*
Fibre-supplemented diet	−0·374	0·286	0·594	0·070	0·090	0·805
Atopobium group						
Normal diet (baseline)	0·415	0·233	−0·238	0·5074	−0·327	0·356
Fibre-free diet	−0·077	0·833	−0·130	0·720	0·189	0·601
Normal diet (baseline)	−0·090	0·805	0·125	0·730	−0·246	0·492
Fibre-supplemented diet	0·180	0·619	−0·640	0·046*	0·273	0·445
Bifidobacteria						
Normal diet (baseline)	−0·286	0·424	0·258	0·471	0·070	0·848
Fibre-free diet	0·486	0·155	−0·400	0·252	−0·160	0·659
Normal diet (baseline)	−0·371	0·291	0·263	0·463	0·206	0·568
Fibre-supplemented diet	0·264	0·460	−0·255	0·477	−0·127	0·726
Ruminococci						
Normal diet (baseline)	0·146	0·688	−0·536	0·111	0·051	0·889
Fibre-free diet	−0·609	0·062	0·422	0·225	0·665	0·036*
Normal diet (baseline)	0·362	0·304	−0·485	0·155	0·546	0·102
Fibre-supplemented diet	0·361	0·305	−0·132	0·717	0·003	0·994
Roseburia intestinalis group						
Normal diet (baseline)	−0·600	0·067	0·289	0·417	0·307	0·389
Fibre-free diet	−0·503	0·139	0·284	0·426	0·284	0·427
Normal diet (baseline)	−0·283	0·429	0·147	0·686	0·266	0·458
Fibre-supplemented diet	−0·125	0·731	0·103	0·777	0·084	0·818
Enterobacteriaceae						
Normal diet (baseline)	0·398	0·255	−0·004	0·991	−0·171	0·637
Fibre-free diet	−0·073	0·841	0·138	0·704	−0·165	0·649
Normal diet (baseline)	0·259	0·470	−0·340	0·337	0·670	0·034*
Fibre-supplemented diet	−0·179	0·621	−0·117	0·747	−0·070	0·847

* $P<0·05$.

fibre-supplemented liquid diet also correlates with the reduction in faecal butyrate. Duncan *et al.* have previously shown a correlation between *Roseburia* and butyrate production in obese patients on a carbohydrate-restricted diet; however, in that study the correlation with *F. prausnitzii* was poor[23].

We describe an in-depth analysis of faecal samples from a study of healthy subjects consuming defined liquid diets either devoid of fibre or fibre-supplemented. In the previous report[18], SCFA were measured in the stool samples and all major SCFA (acetate, propionate, butyrate) were reduced following a fibre-free diet, whereas following a fibre-supplemented diet acetate and propionate did not significantly decrease from baseline, whilst butyrate did. However, without the present analysis, and in particular the detection of the decrease in *F. prausnitzii* during both diets, the reduction in butyrate during both diets would have remained unexplained[24]. Although there were also correlations between butyrate and other species, only in *F. prausnitzii* did the correlation occur in both baseline diets and in the dynamic effects analysis. The results for FISH analysis were remarkably reproducible, with bacterial counts similar to the original analysis following long-term storage in ethanol–PBS at −20°C. The present analysis used automated, rather than manual, counting and this may explain the subtle differences in results found here compared with the previous analysis.

There was an increase in proportion of bifidobacteria during the fibre-containing diet, which contained prebiotic fructo-oligosaccharides. This may explain why acetate concentrations were higher compared with during the fibre-free diet, as bifidobacteria are major producers of acetate[18]. Other bacteria responsible for butyrate production, such as *Roseburia* spp., are associated with insoluble fibres and ferment these as an energy source[25]. In addition, ruminococci also attach to dietary fibres[26] and these bacteria also show a trend towards lower numbers during both fibre-free and fibre-supplemented diets.

Table 5. Correlation between the change in proportion of intestinal microbiota (as percentage of total cells) and the change in the proportion of acetate, propionate and butyrate (as percentage of total SCFA) between the standard diet (baseline) and a fibre-free or a fibre-supplemented diet

	Change in SCFA					
	Acetate		Propionate		Butyrate	
Change in microbiota	*r*	*P*	*r*	*P*	*r*	*P*
Bacteroides						
Baseline to fibre-free diet	−0·489	0·151	0·272	0·448	0·597	0·069
Baseline to fibre-supplemented diet	−0·041	0·909	0·185	0·609	0·015	0·967
Eubacterium rectale group						
Baseline to fibre-free diet	−0·358	0·309	0·209	0·563	0·128	0·724
Baseline to fibre-supplemented diet	0·118	0·746	−0·113	0·755	0·519	0·124
Faecalibacterium prausnitzii group						
Baseline to fibre-free diet	−0·103	0·777	−0·106	0·771	0·806	0·005*
Baseline to fibre-supplemented diet	0·671	0·034*	−0·603	0·065	0·749	0·013*
Atopobium group						
Baseline to fibre-free diet	−0·071	0·845	0·162	0·654	0·323	0·362
Baseline to fibre-supplemented diet	0·175	0·629	−0·479	0·161	0·243	0·499
Bifidobacteria						
Baseline to fibre-free diet	0·408	0·242	−0·296	0·407	−0·320	0·368
Baseline to fibre-supplemented diet	0·091	0·803	0·067	0·854	−0·486	0·155
Ruminococci						
Baseline to fibre-free diet	−0·392	0·263	0·526	0·119	−0·277	0·439
Baseline to fibre-supplemented diet	0·245	0·495	−0·202	0·575	0·539	0·108
Roseburia intestinalis group						
Baseline to fibre-free diet	−0·404	0·247	0·268	0·454	−0·121	0·739
Baseline to fibre-supplemented diet	0·147	0·685	−0·099	0·786	0·264	0·462
Enterobacteriaceae						
Baseline to fibre-free diet	−0·332	0·348	0·489	0·152	0·089	0·807
Baseline to fibre-supplemented diet	0·425	0·221	−0·570	0·085	0·304	0·393

* $P<0·05$.

We speculate that the inability of the fibre-supplemented formula to fully maintain the original microbiota might be related to the composition of the insoluble fibres contained within the liquid enteral formula. The fibre-supplemented formula contained 48 % soluble and 52 % insoluble fractions from fructo-oligosaccharides and pea fibre, and it has been shown that different sources of dietary fibre lead to the formation of different SCFA profiles[27]. In pea fibres the strong linkages between uronic acid and xylose protect the xylose from fermentation, whereas in wheat-bran fibres the majority of the xylose molecules are ordered in linear xylans which are preferentially degraded. The fermentation of xylose provides a preferential substrate for the production of butyrate; therefore wheat-bran fibre leads to a higher production of butyrate than pea fibre[27]. Whilst the reduction in *Roseburia* spp. and *F. prausnitzii* during the fibre-free diet may be caused by the absence of fibre, their reduction during the fibre-supplemented diet may relate to the presence of a fibre that does not support proliferation of *Roseburia* and *F. prausnitzii*. A recent study showed that inulin increased the numbers of both bifidobacteria and *F. prausnitzii*[28]. However, we cannot confirm this increase in *F. prausnitzii* with the data from the present study.

There was a reduction in faecal weight and frequency during both liquid diet regimens. This, together with the lower concentrations of bacteria and SCFA, reflects an even larger fall in total amounts of bacteria and SCFA. As none of the volunteers reported diarrhoea, a common complication of enteral nutrition, this is not a cause of the reduction in *F. prausnitzii* in these samples[29].

Reduction of the butyrate-producing capacity of the microbiota is likely to be important in both health and in specific disease states. Low amounts of this primary energy source for the colonocytes may result in decreased intestinal barrier integrity. Furthermore, abnormal water excretion in the colon has been observed during enteral feeding which probably contributes to the pathogenesis of diarrhoea[30]. This effect has been shown to be reversed when supplementing SCFA, including butyrate, to the colon[3]. A case study on antibiotic-induced diarrhoea supports the concept that lowered levels of SCFA coincide with the occurrence of diarrhoea[31].

In a recent study on the microbiota of patients with Crohn's disease, those who were still in remission 6 months after surgery were found to have higher mucosal concentrations of *F. prausnitzii* compared with those who had relapsed[32]. Meanwhile, patients with active inflammatory bowel disease have lower faecal concentrations of *F. prausnitzii* than healthy controls[15]. Studies have demonstrated that this bacterial strain has pronounced immunoregulatory effects. These include decreased IL-12 and interferon-γ and increased IL-10 production in peripheral blood mononuclear cells, and marked improvement in inflammation in a murine model of colitis[32]. A significant reduction in *F. prausnitzii*, as shown in the present study, might therefore potentially lead to an increased inflammation and disease activity in Crohn's disease.

Whilst the present analysis relates to luminal, not mucosal, microbiota the present results seem somewhat paradoxical, as we have shown that enteral formulas cause a reduction in *F. prausnitzii*, despite the fact that they are used as primary

treatment in active Crohn's disease. The exact mechanism of effect of enteral formulas in inducing remission in Crohn's disease is not clear, and the role of the mucosal and luminal microbiota requires further research. The present study suggests that this beneficial effect of enteral formula use is probably not mediated by *F. prausnitzii*.

Patients with isolated colonic Crohn's disease, who have no ileal disease, respond poorly to treatment with enteral formulas alone[33]. As *F. prausnitzii* has been found in lower numbers in patients with Crohn's colitis[15], our present results might provide an explanation why patients with isolated colonic Crohn's disease do not respond well to treatment with only enteral formulas, in contrast to patients with isolated ileal Crohn's disease. The mechanisms necessary for inducing remission probably differ for the different disease states and localisation of Crohn's disease. Whereas microbial debulking might prove beneficial in ileal Crohn's disease, the coinciding depletion of colonic *F. prausnitzii* might not be beneficial in isolated colonic Crohn's disease. What is of striking relevance is the positive association between *F. prausnitzii* and butyrate production in the gastrointestinal tract, especially since butyrate may be therapeutic for active ulcerative colitis and Crohn's disease[34]. More studies of the effect of enteral formulas on the microbiota and SCFA, in particular *F. prausnitzii* and butyrate, are required in Crohn's disease.

For future research it is important to include an extensive probe set when investigating effects of the intestinal microbiota on SFCA production with FISH. Furthermore, alternative fibre supplements should be designed to restore the numbers of butyrate-producing bacteria and concentrations of butyrate in the faecal samples; these should be investigated for use in enteral formulas.

Conclusion

We have shown that a diet either devoid of fibre, or a diet supplemented with dietary fibre wherein pea fibre constitutes the main insoluble fraction, is accompanied by lower numbers of bacteria from the *F. prausnitzii* and *Roseburia* groups. The decrease of bacteria from the *F. prausnitzii* group is correlated with lower concentrations of faecal butyrate.

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All authors declare that they have no conflicts of interest.

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