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Bacterial population dynamics and faecal short-chain fatty acid (SCFA) concentrations in healthy humans

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Fermentation products, SCFA, particularly butyrate, are considered a sign of 'good' bowel health but the influence of bacterial population composition and diet on inter-individual difference in metabolites and colonic health is poorly understood. Faecal specimens were collected weekly from eight healthy human volunteers over 12 weeks. Dietary intake was self-reported and ten macronutrient factors were analysed at selected weekly periods. Faecal weight, pH and moisture were recorded, and SCFA concentrations were measured in all samples. From each specimen, DNA was prepared and eubacterial 16S rRNA gene PCR performed. Bacterial population profiles were captured by denaturing gradient gel electrophoresis (DGGE) of PCR products, and multivariate statistical analysis was performed. Faecal weight, pH and moisture varied widely within and between individuals. Average total SCFA concentrations over 12 weeks ranged from 36·9 to 144·4 mmol/kg in 48 h specimens and faecal butyrate concentrations ranged from 1·8 to 48·5 mmol/kg. Two individuals with butyrate concentrations below 10 mmol/kg were considered to be 'low butyrate types' and may represent an at-risk population for bowel health. Dietary fat, sugar and carbohydrate showed weak correlation with SCFA (R −0·612, P=0·015; R 0·607, P=0·016; R 0·610, P=0·016, respectively) and butyrate concentrations (R −0·593, P=0·02; R 0·504, P=0·054; R 0·528, P=0·043, respectively). Multivariate analysis of DGGE bacterial profiles demonstrated concise and repeated grouping of intra-individual samples, but these were combined with distinct inter-individual differences (analysis of similarities $P<0·001$, $R \geq 0·99$) The exact relationship of these SCFA values to the overall bacterial profiles and SCFA-producer bacterial groups was not direct nor linear.

Short-chain fatty acids: Bacterial population dynamics: Healthy humans

The colonic microbiota forms a complex interface between dietary components and colonic health. Our understanding of colonic bacterial population dynamics and their consequent influence on gut disease risks is poor. The human colon typically contains a large and diverse population of bacteria ($>10^{11}$ bacteria/g contents) which play an important role in the normal development of the intestine and provide an important energy source via fermentation of 'resistant' starches[1].

Strictly anaerobic bacteria such as *Bacteroides*, *Bifidobacterium*, *Fusobacterium*, *Clostridium* and *Peptostreptococcus* species are thought to predominate, as measured by both cultural and molecular methodologies[2–4]. Certain genera, such as *Lactobacillus* and *Bifidobacterium*, may indicate a 'healthy' bowel[5,6], whereas others have been linked with an increased colon cancer risk[7]. Considerable variation in the distribution of bacterial species between individuals has been clearly identified[4,8,9] but few of these studies had detailed dietary information. There is little information on how an individual's bacterial population may change over time. Importantly, many enteric bacterial species cannot be readily cultured and may have been omitted from some previous studies.

An important metabolic process of colonic bacteria is their production of the SCFA: acetate, butyrate and proprionate. These SCFA, particularly butyrate, are often absorbed and may be utilised directly as an energy source by colonic epithelial cells[10–12]. Several lines of research have indicated that butyrate may be protective for colonic cell damage. Cell culture studies have indicated that the presence of butyrate at physiological concentrations enhances the growth of normal enterocytes and inhibits that of malignant ones[13,14]. A rising butyrate concentration within the large bowel in a rodent colon cancer model was correlated with increased apoptosis in the aberrant crypt foci in affected colons[15]. Although the causes of the various forms of inflammatory bowel disease complex may be generally related to dysregulated immune responses to commensal bacteria[16,17] distal ulcerative colitis has also been related to SCFA metabolism and/or availability in the colon[18]. Colon cells isolated from distal ulcerative colitis patients had an impaired capacity to oxidise butyrate and faecal butyrate levels were higher in adult and infant colitis patients than in controls[19]. It is therefore possible that a defect in butyrate metabolism occurs in distal ulcerative colitis, which could be overcome through provision of more substrate. SCFA enemas (including butyrate) have been reported to induce remission in colitis-affected patients[20]. Identification of individuals with low faecal SCFA concentrations could also provide a target population for dietary or microbiota modulation or therapeutics. The SCFA are produced solely via bacterial

Abbreviation: DGGE, denaturing gradient gel electrophoresis.

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anaerobic fermentation pathways in the colon, particularly the proximal portion. However, the number and extent of SCFA and bacterial studies on colon samples from cadavers or colostomy patients have been limited[21,22], and the biology of SCFA production along the length of the colon largely remains an interpolation of faecal studies in man.

This preliminary study explores bacterial population dynamics and faecal SCFA concentrations in eight healthy human volunteers consuming their regular diet. The application of new molecular techniques to biological samples enables a more comprehensive analysis of colonic bacterial species and their relationship to diet and health.

Experimental methods

Participants and sample collection

A 12-week bowel health study was designed to examine enteric bacterial microbiota and SCFA concentrations in eight healthy participants consuming their regular diets. Six unrelated free-living volunteers and one married free-living couple (coded as PH01 to PH 08) were recruited to the trial from one city of one million residents. Approval was obtained from the CSIRO Health Sciences and Nutrition Human Ethics Committee and informed, written consent obtained from each volunteer. Subjects with a history of bowel disorder, such as irritable bowel syndrome, Crohn's disease or colorectal cancer, were excluded from the study. Subjects were required to be antibiotic-free for at least 3 months prior to commencement of the trial. Volunteers were required to complete a food diary, recording all food, drink or medication consumed, over 2 separate weeks during the 12-week study. The macronutrient composition of each volunteer's diet was calculated using daily food diaries and average daily nutrient intakes based on the nutrient composition of each food or beverage item per unit weight. These figures were calculated by reference to Australian food tables using the CSIRO dietary analysis system[23]. The Australian food nutrient data were supplemented by British, US or manufacturer's data where Australian data are missing[24].

Fresh faecal specimens were collected over a 48 h period once a week for 12 weeks. In addition, for 1 week of the study bowel actions were collected daily. Each bowel movement was packaged separately and frozen directly after collection, and stored at −20°C. Specimens were transported frozen to the central laboratory. Faecal samples were taken to be an accurate reflection of colonic organisms in number and identity[25].

Specimens were defrosted at room temperature and all processing was performed under anaerobic conditions. Each stool mass was recorded prior to combining the 48 h collection from each volunteer (henceforth referred to as the specimen). The specimen was thoroughly mixed by hand homogenisation and the pH measured. Faecal aliquots (samples) were taken for SCFA analysis, DNA extraction and faecal moisture calculation.

SCFA measurement

SCFA concentrations in faecal specimens were measured according to a modified method of Patten *et al.* [26]. Briefly, duplicate 1 g faecal specimens were prepared for SCFA distillation by addition of 3 × volume for weight of 1·68 mM-heptanoic acid, pH 7, mixed and centrifuged at 3000 rpm for 10 min at 5°C. Supernatant (150 μl) was distilled under vacuum and 60 μl of the distillate were transferred to a glass GC vial prior to loading on the Agilent 6890N Network gas chromatograph system with automatic loader/injector. The GC column was a Zebron ZB-FFAP (Phenomenex, Lane Cove, NSW, Australia), length 30 m, internal diameter 0·53 mm, film thickness 1 μm. The GC was programmed to achieve the following run parameters: initial temperature 90°C, hold 0·5 min, ramp 20°C/min, final temperature 190°C, total run time 8·0 min. Gas flow 7·7 ml/min splitless to maintain 3·26 psi column head pressure, septum purge 2·0 ml/min.

Calibration standards were prepared to give a mixture of the following concentrations of acids (mM): 26·22 acetic, 19·86 propionic, 3·24 isobutyric, 16·32 butyric, 5·40 isovaleric, 5·46 valeric, 4·74 caproic and 5·04 heptanoic. This standard mix (0·2 μl) was used to calculate retention times and create a standard plot. Additional standards were included in each GC run of samples at five sample intervals to maintain calibration; 0·2 μl of each subsample distillate was analysed by GC and an integrator was used to plot the curve of the standards and provide the concentration of acids present in mM.

The total SCFA (the sum of acetate, propionate and butyrate values) excreted in a 48 h stool collection (pooled values or total output) were calculated using total faecal output (in g) and faecal moisture values.

DNA extraction and purification

Extraction of DNA from duplicate 0·1 g faecal samples was performed using the QIAmp® DNA Stool Mini Kit (Qiagen, Hilden GmbH, Germany), following the supplied protocol for isolation of bacterial DNA from stool. Quality, high molecular weight, DNA was determined on 1 % (w/v) agarose gels containing 10 μg/ml ethidium bromide and visually assessed under UV transillumination.

Denaturing gradient gel electrophoresis–PCR

Approximately 100–200 ng of genomic DNA were used as template for denaturing gradient gel electrophoresis (DGGE)–PCR. Universal bacterial 16S rRNA gene primer sets were used to amplify regions of the 16S rRNA gene. They were comprised of forward primer 907f (5′-AAACTCAAA GGAATTGACGG-3′[27]) and GC-clamped reverse primer 1392rc (5′-CGCCCGCCGCGCCCCCGCCCGGCCCGCCGCCCCCGCCCCACGGGCGGT GTGTRC-3′[28]) resulting in a PCR product size of about 500 bp. A mixture of diluted reamplified DGGE bands was used as a positive control template for PCR–DGGE.

Amplification of isolated bacterial 16S rRNA gene was performed using the primers 10f and 1492r[29] resulting in a product size of about 1500 bp. Each PCR contained one unit of *Taq* DNA polymerase (Promega, Madison, WI, USA), 2·5 μl 10×reaction buffer (Promega), 1·5 μl 25 mM-$MgCl_2$, and 10 mM-deoxynucleotide triphosphates (dNTP), genomic DNA and 12·5 pmol of each primer, in a total volume of 25 μl. PCR cycling parameters followed a touch down protocol of twenty-nine cycles on a Hybaid PCR Express thermal

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cycler (Hybaid, UK), and incorporated 0·5°C steps from 65 to 55°C with 2 min 72°C extension and 1 min 95°C denaturation steps, with a final 4 min 72°C extension step.

Denaturing gradient gel electrophoresis analysis

DGGE was performed using the D-code Universal Mutation Detection System (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's method for perpendicular gels. PCR product (15 μl) was loaded per lane and electrophoresed on a 16 cm × 15 cm 6 % acrylamide gel containing a 35–80 % denaturing gradient for 16 h at 60°C. Denaturation solutions were made by combining 0 % (6 % v/v acrylamide in 1 × TAE buffer (40 mM-Tris-acetate,1 mM-disodium EDTA (pH 8·0))) and 100 % (6 % v/v acrylamide, 40 % v/v deionised formamide, 42 % w/v urea in 1 × TAE buffer) denaturation solutions. Gels were stained for 30 min in 100 ml 1 × Sybr-gold (Molecular Probes, Eugene, OR, USA) in 1 × TAE and destained for 5 min in 100 ml Milli-Q H_2O. Images were digitally captured under 302 nm UV transilluminatation using the DigiDoc apparatus and Software (Bio-Rad Laboratories). For comparison between gels the positive control PCR reaction was run in the outside lanes and was used to standardise fragment migration when comparing between gels.

Data analysis and statistics

Elementary statistical analysis (mean, standard deviation and CV) was applied to the data. Correlations were calculated using Pearsons linear regression model (R) and coefficient of determination (R^2). Multivariate analysis (multidimensional scaling and analysis of similarities)[30,31] of DGGE banding patterns was performed using the Primer 6 package (PRIMER-E Ltd, Plymouth, UK). DGGE bands migrating to the same position in separate lanes were considered for the purpose of analysis to represent the same sequence.

Results

Study subjects

The ages of the subjects (three men, five women) ranged from 31 to 59 years. BMI ranged from 23·1 to 28 kg/m^2; none of the volunteers were obese. All subjects consumed their regular diets for the duration of the study. None of the volunteers were smokers. One subject (PH01) withdrew at week 8 of the trial due to travel commitments. Occasional samples were not collected at week 1 (PH08), week 6 (PH01), week 10 (PH02), week 12 (PH02, PH04, PH05) due to personal or travel commitments.

Dietary analysis

Food diaries were completed during weeks 4 and 9 (PH01–PH05) and weeks 7 and 10 (PH06–PH08). The daily macronutrient composition, comprising forty dietary components, consumed by each volunteer during two separate weeks was calculated using CSIRO Dietary Analysis Software[23] and data on a subset of ten major dietary constituents, energy, water, protein, fat, carbohydrate, complex carbohydrate, sugar, fibre, total NSP and alcohol, are presented in Table 1, and the average daily values reflect the inconsistency and

Table 1. Macronutrient composition of volunteer diets: average daily intake of nine major dietary nutrients during 2 separate weeks of the study

	Energy (kJ/d)		Water (g/d)		Protein (g/d)		Fat (g/d)		CHO (g/d)		Complex CHO (g/d)		Sugars (g/d)		Fibre (g/d)		Total NSP (g/d)		Alcohol (g/d)	
Volunteer	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV	Mean	CV
PH01	7868	4·9	2538	11	85	5·3	58	2·4	195	4·6	105	6	88	2·2	24	3·2	17	0·7	34	20·2
PH02	7865	4·2	2167	5·9	89	6·5	79	12·9	200	3·8	87	2·1	109	7·4	21	4·3	16	2·4	3	47·1
PH03	8441	8·7	1265	6·3	84	7·1	94	28	214	4·8	107	6·8	102	3·6	32	0·8	25	0·6	0	0
PH04	6958	12·5	2305	6·7	72	11·6	55	10	194	13·4	64	29·5	130	5·9	14	23·3	9	21·8	16	17·4
PH05	6970	24·1	1724	5·8	77	32·3	62	44	167	15·9	101	13·5	66	19·2	16	9·4	11	4·1	21	23·1
PH06	8937	9·6	2856	0·6	93	10·4	65	3·2	261	19·7	114	28·1	145	13·2	17	7·2	12	6·9	24	23·4
PH07	9473	8·8	2102	8·8	88	26·7	99	6·1	248	7·8	125	17·7	118	1·4	27	9·9	21	10·5	6	68·2
PH08	7887	11·2	2035	3·7	93	12·5	109	15	100	7·6	54	24·7	40	6·5	16	3·2	11	16	22	33

CHO, carbohydrates; CV, percentage CV between the 2 weeks.

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variety of volunteers' regular diets. Average energy varied from 6970 to 8441 KJ/d in females and 6958 to 9472 KJ/d in males. Alcohol intake had the greatest variation between weeks for most volunteers and the CV ranged from 0 (non-consumer) to 68·2 %. Daily patterns of nutrient intake were variable, and different patterns of eating could be observed (e.g. increased intake of dietary components associated with weekends in some volunteers) (data not shown).

Faecal specimens

Total faecal output over 48 h was calculated for each volunteer in the 12-week study period (Table 2), and collections varied from 0 to 579 g. Daily faecal output (24 h) ranged from 0 to 377 g (Table 3). Faecal output was greater in males than females for both average weekly and average daily collections but did not reach significance (ANOVA F 2·33, $P=0·178$; F 4·94, $P=0·068$, respectively). An irregular daily pattern of faecal output was observed in women, and for each female participant bowel movements were absent on 2 or more days of the week. Frequency of bowel movements ranged from 0 to 5 over 48 h, and per day averaged 1·02 for males and 0·90 for females ($P \geq 0·05$). Faecal pH ranged from a minimum pH 5·7 to a maximum of pH 8, averaging 7·2 for females and 6·9 for males (ANOVA $P=0·144$; data not shown). Faecal moisture ranged from 61 to 85 %, averaging 76 % for males and 69 % for females and variance between repeat specimens for each volunteer was small (CV $<$ 8·3 %; data not shown). Moisture differences between genders approached significance, ANOVA $P=0·053$, with male samples more moist than female samples. Faecal moisture was positively correlated with total faecal output (48 h collections; Pearson's correlation R 0·557, $P=0·00$). Faecal pH values were negatively correlated with both faecal output (R −0·222, $P=0·04$) and faecal moisture (R −0·507, $P=0·00$).

SCFA measurement

SCFA were measured in a total of eighty-six weekly faecal specimens. Faecal concentrations of SCFA in 48 h collections ranged from 21·2 to 186·6 mmol/kg and average values for each volunteer over 12 weeks varied from 36·9 to 144·4 mmol/kg, demonstrating inter-individual variation (Table 4). Intra-individual variation in total SCFA concentration was observed over 12 weeks and CV ranged from 11·8 to 39·1 % (Table 4). Total SCFA output over 48 h was more variable than SCFA concentration (Table 4), and reflects differences in faecal output (and moistures) in both inter- and intra-individual specimens. Faecal butyrate concentrations showed similar patterns but were more variable than total SCFA (Table 5). Concentrations ranged from 1·8 to 48·5 mmol/kg, and total butyrate output over 48 h ranged from 0·2 to 12·9 mmol. Scrutiny of standard deviation reveals greater variation in SCFA values between individuals than within individuals, indicating a consistent difference between individuals.

Overall, faecal output was positively correlated with SCFA output in weekly specimens (R 0·848, $P=0·00$; note that faecal output values are used to calculate SCFA output), however, there was only weak correlation with SCFA concentration (R 0·392, $P=0·00$). These patterns of correlation held true when

Table 2. Total faecal output (g) collected over a 48 h contiguous period each week for 12 weeks

Volunteer	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Mean	SD
PH01	297	488	323	381	305	0	388	127	NA	NA	NA	NA	289	155
PH02	340	142	136	68	89	137	97	177	256	0	12	NA	132	100
PH03	387	400	302	238	222	398	382	69	134	513	399	116	297	139
PH04*	378	407	579	536	287	439	456	414	407	512	418	NA	439	80
PH05	235	148	349	303	364	224	184	130	184	216	229	0	214	99
PH06*	60	383	282	159	138	245	154	218	156	236	179	209	202	82
PH07*	366	349	352	410	247	406	239	138	226	439	333	278	315	90
PH08	NA	227	193	80	97	143	153	42	155	220	69	68	132	65

NA, not available.
*Male volunteer.

Table 3. Daily (24 h) faecal output recorded over 7 consecutive days

Volunteer	Mon	Tue	Wed	Thur	Fri	Sat	Sun	Mean	SD
PH01	230	0	305	261	192	0	0	141	136
PH02	0	89	0	148	103	227	99	95	80
PH03	0	52	170	377	43	93	0	105	133
PH04*	158	169	119	244	175	117	210	170	46
PH05	126	155	209	0	0	121	35	92	81
PH06*	213	219	89	90	85	190	0	127	82
PH07*	92	132	265	68	99	124	196	139	67
PH08	36	99	69	0	92	0	10	44	43

* Male volunteer.

data sets for individuals were examined. Faecal moisture content was weakly associated with SCFA output and concentration (R 0·637, P=0·00; R 0·529, P=0·00, respectively). A weak negative correlation was observed between pH and SCFA output and concentration (R −0·479, P=0·00; R −0·679, P=0·00), respectively.

SCFA and dietary macro-constituents

To partially compensate for intestinal transit time dietary data was derived from 24 h prior to faecal collection and the first 24 h of the 48 h faecal collection period. Of nine dietary macro-constituents analysed, only fat, sugar and carbohydrate intake correlated with SCFA concentration, and only fat and sugar correlated with SCFA output (Table 6).

Bacterial population dynamics

DNA was extracted from a total of eighty-six weekly specimens and forty-four daily specimens, and DNA quality was confirmed by agarose gel electrophoresis. Multivariate analysis of DGGE banding patterns from individual faecal samples demonstrated stable bacterial populations specific to each individual (Figs. 1 and 2). Clear inter-individual differences were observed in DGGE profiles of faecal bacterial populations (Figs. 1 and 3). Analysis of similarities analysis of total DGGE banding patterns confirmed significance of inter-individual differences for the dominant bacterial groups detected by PCR–DGGE ($P<0·001$, $R \geq 0·99$).

Between sixteen and twenty-five distinct bands were detected in each weekly faecal specimen and some variation in the intensity of individual DGGE bands was observed between intra-individual specimens, as illustrated in Fig. 1.

Discussion

Examination of the faecal bacterial eco-system in eight humans over a 12-week period generated detailed information on its bacterial population dynamics and on its metabolic products. In order to achieve a realistic view of possible variability between free-living adults, we did not impose a controlled diet on volunteers, but recorded dietary intake and composition as a study variable. By combining molecular analysis of bacterial populations with physiological measures we further demonstrated the complexity of the colonic environment and highlighted the potential of a multidisciplinary approach to understanding inter-relationships which are likely to have a bearing on human colonic health.

The present studies of the volunteers' colonic bacterial populations confirm their clear variability between individuals on omnivorous Western diets, as indicated in previous studies of free-living human subjects and other omnivores[32–35]. The DGGE analysis visualised between sixteen and twenty-five distinct bands, out of an estimated 400–500 species, per faecal sample with only three DGGE bands common to all volunteers. Besides this expected variability between individuals, we also found that the genera comprising each individual's microbiota changed very little over the study. It is yet to be clearly established whether a consistent change in diet under controlled conditions would give rise to bacterial population shifts. The 12-week period chosen for the present study was considered to reflect a likelihood of reliable extrapolation to longer periods, possibly semi-annual. In other words each individual carried a distinct but stable microbiota. This occurred despite all the temporal variations in dietary intake (energy, fat, fibre, etc) associated with a Western diet. This microbiota stability has been suggested in occasional less detailed studies[9,33], but we consider the present study to be more wide-ranging. This stability also confirms widespread notions of the 'colonisation resistance' effect of the resident colonic microbiota to incoming novel bacteria[36,37].

The faecal SCFA concentrations in the volunteers were broadly similar to those noted in other Western citizens in previous single-sample studies[22]. However, in contrast to the bacterial microbiota, we established that the volunteers' SCFA concentrations were widely variable over the 12-week study period. This variability was noted both within each healthy adult repeatedly sampled and between adults (including between each individual in a marriage). Despite this variability, we were able to stratify individuals within their ranges of SCFA concentrations observed. Two individuals (PH02 and PH08) consistently had low concentrations of total SCFA (average of 56·4 and 36·9 mmol/kg) and butyrate (average of 7 and 6 mmol/kg) in their faeces, as compared to their same-city cohorts. The remaining individuals had higher and more variable concentrations with averages typically over 100 (total SCFA) and 20 mmol/kg (butyrate). It is of interest that some individuals (25 % of the study group) may have consistently low concentrations of SCFA in their faeces and could represent an at-risk population for bowel health. It is possible that these individuals may have altered production and/or absorption of SCFA within their colon. Bacterial metabolic activity, including SCFA production, is thought to be more intense in the proximal colon where the environment is more fluid and substrate availability greater[25,38]. It would therefore

Table 4. Total SCFA (acetate, propionate and butyrate) concentrations and total output from faecal specimens collected weekly over 12 weeks

Volunteer	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Mean	SD
Total SCFA concentration (mmol/kg)														
PH01	84·4	148·9	119·9	94·5	69·2	N/S	72·7	115·3	NA	NA	NA	NA	100·7	28·8
PH02	62·2	94·8	45·2	66·7	86·6	46·1	52·2	50·8	39·1	N/S	20·7	NA	56·4	22·1
PH03	85	77·3	111·1	56	44·2	46·5	76·9	66·5	112·1	90·4	98·7	97·2	80·2	23·3
PH04	112·3	36·7	112·1	132·2	102·5	105·7	109·2	116·7	112·9	75·6	97·8	NA	101·2	25·5
PH05	129·1	90	123·8	140·2	123·3	186·6	84·7	95·3	92·1	68·6	51·7	N/S	107·8	37·6
PH06	133·1	118·6	101·1	110	119·2	132·1	109·6	99·4	119·1	104·2	127·4	91·4	113·8	13·4
PH07	163·6	118·4	147·8	181	133·6	132·8	136	157·9	156	144·9	155·8	105·4	144·4	20·6
PH08	NA	28·7	21·2	38·1	25·5	58·5	27·1	48·6	33	37·9	45·5	45·3	36·9	11·1
Total SCFA output over 48 h (mmol)														
PH01	17·7	52·3	24·7	25·6	12·8	N/S	17·9	9·1	NA	NA	NA	NA	22·9	14·2
PH02	14·7	8·5	4	2·9	5·5	4·4	3·4	6·1	7·2	N/S	0·2	NA	5·7	4·0
PH03	23·6	23·3	25·2	9·1	6·6	13·4	21·6	3·1	10·6	34·8	30·6	7·6	17·5	10·3
PH04	33·5	11·5	51·4	54·9	24·1	39·3	37·2	39·5	35·4	31·5	31·5	NA	35·4	11·8
PH05	22·2	10·7	32·9	32·4	33·8	33·3	10·6	9·3	12·4	11·3	8·4	N/S	19·8	11·2
PH06	6·1	34·7	20·4	13·4	12·4	23·9	12·6	15·8	13·4	18·9	16·2	13·2	16·7	7·2
PH07	44·4	31·2	38·4	54·5	24·2	39·5	24·6	15·6	26·4	47·4	41·5	20·9	34·0	11·9
PH08	NA	4·4	2·7	1·9	1·7	5·3	2·8	1·3	3·5	5·6	2·1	2·1	3·0	1·5

NA, not available; N/S, no sample.

Table 5. Butyrate concentrations and total output from faecal specimens collected over 12 weeks

Volunteer	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Mean	SD
Butyrate concentration (mmol/kg)														
PH01	18·7	39·1	36·1	20·1	17·8	N/S	18·4	38·3	NA	NA	NA	NA	26·9	10·3
PH02	7·8	15·9	4·1	6·4	11·5	5·2	5·8	7·2	4·2	N/S	1·8	NA	7·0	4
PH03	20·2	15·9	22·4	11·9	8·6	12·6	14·7	14·6	27·8	17·8	25·2	22·7	17·9	5·8
PH04	26·8	4·4	28·1	30·5	20·8	17·6	30·1	20·5	24·9	17·5	18·9	NA	21·8	7·5
PH05	26·4	25	28·1	30·4	28·6	48·5	20·1	22·5	21·4	16·3	8·5	N/S	25·1	10
PH06	27·8	26·8	23·4	22·1	28·7	28·4	18·6	18	26·2	18·3	27·5	19·2	23·8	4·3
PH07	33·3	27·1	39·5	44·6	32·1	25·3	29·3	29·9	30·1	27·2	32·8	22·9	31·2	6
PH08	NA	4·7	3·3	6·5	3·9	9·8	4·3	7·2	4·6	6·6	7·5	7·4	6·0	2
Butyrate output over 48 h (mmol)														
PH01	3·9	13·7	7·4	5·5	3·3	N/S	4·5	3	NA	NA	NA	NA	5·9	3·8
PH02	1·8	1·4	0·4	0·3	0·7	0·5	0·4	0·9	0·8	N/S	0	NA	0·7	0·6
PH03	5·6	4·8	5·1	1·9	1·3	3·6	4·1	0·7	2·6	6·8	7·8	1·8	3·9	2·3
PH04	8	1·4	12·9	12·7	4·9	6·5	10·2	6·9	7·8	7·3	6·1	NA	7·7	3·3
PH05	4·5	3	7·5	7	7·8	8·6	2·5	2·2	2·9	2·7	1·4	N/S	4·6	2·7
PH06	1·3	7·8	4·7	2·7	3	5·1	2·1	2·9	2·9	3·3	3·5	2·8	3·5	1·7
PH07	9	7·1	10·3	13·4	5·8	7·5	5·3	3	5·1	8·9	8·7	4·6	7·4	2·9
PH08	NA	0·7	0·4	0·3	0·3	0·9	0·4	0·2	0·5	1	0·4	0·3	0·5	0·3

NA, not available; N/S, no sample.

Table 6. Correlation values (*R*) for dietary macroconstituents and SCFA concentration and output

	Dietary component								
	Energy (kJ)	Water (ml/d)	Protein (g/d)	Fat (g/d)	Sugar (g/d)	CHO (g/d)	Complex CHO (g/d)	NSP (g/d)	Fibre (g/d)
SCFA concentration									
Total SCFA (mmol/kg)									
R	−0·001	0·02	0·046	−0·612	0·607	0·610	0·490	−0·206	0·142
P	0·998	0·944	0·871	0·015	0·016	0·06	0·064	0·468	0·614
Butyrate (mmol/kg)									
R	−0·067	−0·124	−0·052	−0·593	0·504	0·528	0·444	−0·173	−0·129
P	0·812	0·661	0·855	0·02	0·054	0·043	0·097	0·537	0·647
SCFA output									
Total SCFA (mmol/48 h)									
R	−0·257	0·289	−0·18	−0·611	0·495	0·248	0·021	−0·287	−0·232
P	0·355	0·296	0·490	0·016	0·061	0·373	0·941	0·30	0·405
Butyrate (mmol/48 h)									
R	−0·279	0·223	−0·227	−0·622	0·495	0·250	0·027	−0·283	−0·229
P	0·314	0·425	0·415	0·013	0·065	0·369	0·925	0·307	0·411

CHO, carbohydrates.

be valuable to link SCFA faecal content to distal colon cancer or other abnormalities in future studies.

The co-analysis of self-recorded dietary composition and faecal SCFA revealed only weak correlations with dietary carbohydrate, fat and sugar intake. Carbohydrates, particularly in the form of resistant starches, provide fermentable substrate to the colon and by-products of this fermentation include SCFA[39]. The correlations of faecal SCFA with dietary fat or sugar content may reflect the variable western patterns of eating. Dietary fat consumption was negatively associated with faecal SCFA concentrations, however, this effect was weak and the actual influence of fats on SCFA production remains unclear. Dietary sugar consumption strongly correlated with carbohydrates (*R* 0·77) and may not have been an independent variable. Simple sugars are predominantly digested and absorbed in the small intestine. Alcohol intake varied between participants but was not associated with SCFA values. Further studies involving more volunteers consuming controlled diets would be needed to further explore these co-relations. Dietary complex carbohydrates, including resistant starches and fibre, such as bran, are known to affect gut transit time. Dietary carbohydrates have been linked with increased faecal SCFA concentration or output[40], but in the present study we found no clear relationship.

The influence of the composition of the colonic bacterial population on the relationship between diet and SCFA status was only partially addressed. Each of the volunteers demonstrated a distinct and stable individual DGGE profile of the various bacterial species present. Interestingly, the two individuals with the lowest SCFA concentrations, PH02 and PH08, were located adjacent to each other on multidimensional scaling plot analysis of these DGGE profiles (see Fig. 3). It is therefore possible that particular and distinct colonic bacterial populations may be linked to functional production of SCFA. This relationship may not be direct or linear, and requires considerable further characterisation with respect to individual

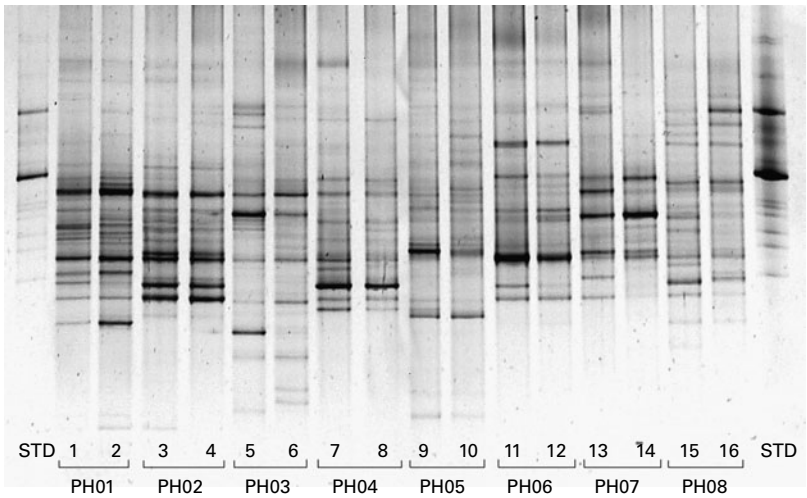

Fig. 1. Denaturing gradient gel electrophoresis (DGGE) profiles of faecal bacterial populations derived from faecal samples from two separate and distant weeks for each volunteer. The negative photographic image of a DGGE gel (formamide–urea gradient 20–55 %) demonstrates variation in community structure between human volunteers (PH01–PH08). Standard tracks (STD) were included to aid band alignment.

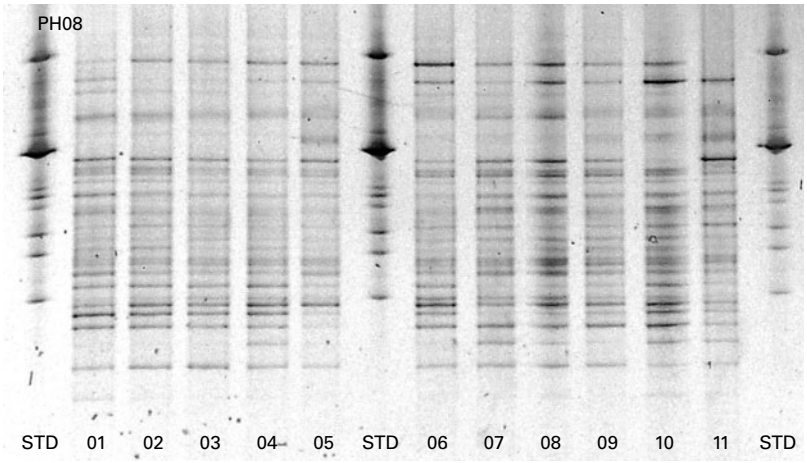

Fig. 2. Denaturing gradient gel electrophoresis (DGGE) profiles of faecal bacterial populations derived from faecal samples collected from one volunteer (PH08) over 11 weeks. A relatively stable pattern is observed. Standard tracks (STD) were included to aid band alignment.

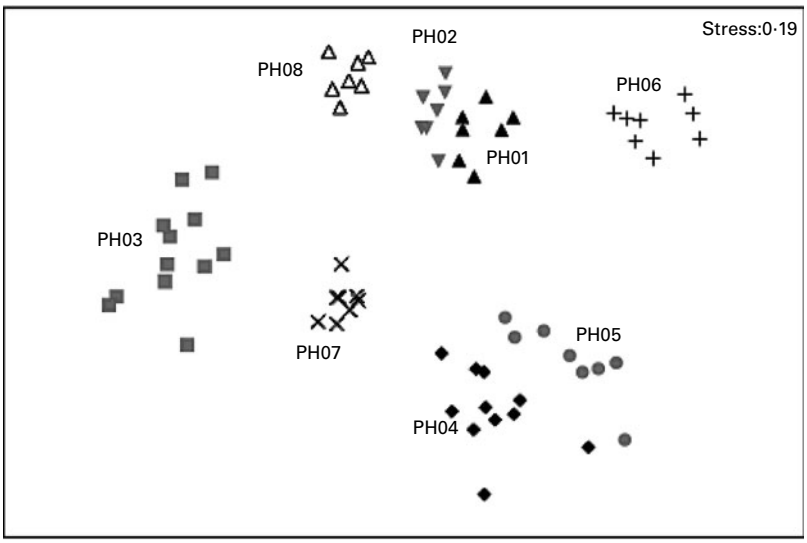

Fig. 3. Multidimensional scaling plot showing the relative similarities of bacterial community structure based on denaturing gradient gel electrophoresis (DGGE) banding patterns between faecal samples. Each volunteer is represented by a different symbol (as annotated on the plot) and data points represent the eubacterial PCR–DGGE banding patterns from a single specimen at a single time-point.

genera and their SCFA competency or redundancy. As information on colonic bacteria proceeds, more specific molecular approaches may allow analysis of functional genes and targeting groups containing butyrate production. Targeted dietary changes may be envisaged to drive microbial composition to a more competent butyrate state or other desired outcome.

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