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Dominant and diet-responsive groups of bacteria within the human colonic microbiota

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Abstract

The populations of dominant species within the human colonic microbiota can potentially be modified by dietary intake with consequences for health. Here we examined the influence of precisely controlled diets in 14 overweight males. Volunteers were provided successively with a control diet, diets high in resistant starch (RS) or non-starch polysaccharides (NSP), and a reduced carbohydrate weight loss (WL) diet, over 10 weeks. Analysis of 16S rRNA sequences in stool samples of six volunteers detected 320 phylotypes (defined at >98% identity) of which 26, including 19 cultured species, each accounted for >1% of sequences. Although samples clustered more strongly by individual than by diet, time courses obtained by targeted qPCR revealed that "blooms" in specific bacterial groups occurred rapidly following a dietary change. These were rapidly reversed by the subsequent diet. Relatives of *Ruminococcus bromii* ("R-ruminococci") increased in most volunteers on the RS diet, accounting for a mean of 17% of total bacteria compared with 3.8% on the NSP diet, while the uncultured *Oscillibacter* group increased on the RS and WL diets. Relatives of *Eubacterium rectale* increased on RS (to mean 10.1%) but decreased, along with *Collinsella aerofaciens*, on WL. Inter-individual variation was marked, however, with >60% of resistant starch remaining unfermented in two volunteers on the RS diet, compared to <4% in the other 12 volunteers; these two individuals also showed low numbers of R-ruminococci (<1%). Dietary non-digestible carbohydrate can produce marked changes in the gut microbiota, but these depend on the initial composition of an individual's gut microbiota.

Introduction

The remarkable diversity of the human colonic microbiota at the level of bacterial species and phylotypes has become apparent from 16S rRNA-based analyses. Not only are hundreds of phylotypes typically estimated to be present in the human colonic microbiota from a given faecal sample (Suau *et al.*, 1999; Eckburg *et al.*, 2005) but samples from different individuals have been reported to show limited overlap in the phylotypes present (Ley *et al.*, 2006; Turnbaugh *et al.*, 2008). A recent study (Tap *et al.*, 2009) however has indicated that certain phylotypes occur more commonly than others among the dominant faecal bacteria of different individuals. Identifying the dominant bacterial species that colonise the large

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intestine and the extent to which these are influenced by diet and host factors is of key importance in uncovering the impact of the colonic microbiota upon human health (Flint *et al.*, 2007; Sokol *et al.*, 2008).

A few studies to date have examined temporal changes, and these suggest a degree of stability in the colonic microbiota of individuals consuming their normal diets (Franks *et al.*, 1998; Zoetendal *et al.*, 1998; Costello *et al.*, 2009). On the other hand, little is known about the impact of dietary change upon microbial community composition. There is evidence that dietary supplementation with prebiotics such as fructo-oligosaccharides and inulin can promote specific groups of bacteria, including bifidobacteria (Bouhnik *et al.*, 2004; Ramirez-Farias *et al.*, 2009). It has also been shown that reductions in total carbohydrate content, in weight loss diets for obese subjects, have major effects upon the composition and metabolic outputs of the bacterial community in the colon (Duncan *et al.*, 2007; Duncan *et al.*, 2008; Brinkworth *et al.*, 2009). These changes are assumed to reflect the fermentation of non-digestible carbohydrate components (mainly non-starch polysaccharides (NSP), resistant starch (RS) and certain oligosaccharides) that reach the large intestine. The impact upon the colonic microbiota of controlled changes in the main types of non-digestible (ND) carbohydrate normally present in the diet (RS and NSP) has not however been examined in any detail. The ND carbohydrate content of the human diet is considered to influence health. For example, diets high in RS have been shown to benefit insulin sensitivity, possibly mediated via bacterial fermentative activity in the colon (Robertson *et al.*, 2005). Diets containing RS and NSP offer potential benefits in prevention of colorectal cancer through the delivery of fermentation acids, in particular butyrate, to the distal colon (Duncan *et al.*, 2007; McIntyre *et al.*, 1993). Microbial breakdown of NSP also releases bound phytochemicals into the colon (Gill & Rowland, 2002). These health benefits may be particularly important in obese and overweight subjects who are at increased risk of developing colorectal cancer and diabetes (Polednak, 2003; Cani *et al.*, 2007).

The present study asks to what extent changes in the major type of ND carbohydrate in the diet influence the dominant bacterial phylotypes present in an individual. It also explores the dynamics of such changes, including their reversibility, for the first time through the use of detailed time courses and a cross-over design. A necessary supplementary question is whether the same dietary change elicits similar changes in the gut microbiota of different individuals. Our results reveal rapid and marked changes in the colonic microbiota of a group of overweight volunteers following a shift in the main type of ingested fermentable carbohydrate (resistant starch or non-starch polysaccharide). We also find that these changes can be highly specific to the individual, with potentially important implications for the design of fibre-enriched diets.

Materials and Methods

Volunteer recruitment

Volunteers were initially recruited by newspaper advertisement. Inclusion criteria were for males with a BMI >27 kg/m^2, waist circumference >102 cm and fasting plasma glucose concentration >6.0 mmol, as features indicating metabolic syndrome. They underwent a medical examination and their general practitioner was contacted to confirm medical and medication history. None of the subjects had a history of gastrointestinal disease or disturbance, or took antibiotics, either in the six months leading up to the study or during the study. Two of the original 16 subjects left the study for reasons unconnected with the protocol. The remaining 14 volunteers had a mean age of 54 y (range 27-73) with a mean body mass index (BMI in kg/m^2) of 39.4 (range 27.9-51.3) (Table S1). The study was approved by the North of Scotland Research Ethics Service and all volunteers provided informed, written consent.

Experimental design and dietary regime

The volunteers were weight stable (less than 3 kg change in the past 4 months) prior to entry on the trial. Over the first seven weeks intakes were provided at energy maintenance as 1.4-1.5 × measured resting metabolic rate for each individual. The final intervention period consisted of high protein weight loss diet for an additional three weeks (Table S2, Fig. S1). The initial maintenance diet (M diet) comprised protein:carbohydrate:fat % as 13:52:35 of metabolisable energy (ME) and 27.7 g/d non-starch polysaccharide, provided for 7 days. Subjects were then provided with fixed intakes of two diets, which consisted of either a high resistant starch (RS diet) or a high non-starch polysaccharide diet (NSP diet) each supplied for three weeks in a randomised cross-over design (Fig. S1). The RS diet contained added type III resistant starch while the NSP diet intake contained added wheat bran. All meals were of the same energy density (5.5 MJ/kg) and daily intakes were recorded by weigh-back after each meal (Table S2). Daily macronutrient intakes were calculated using the Windiet software program (Robert Gordon University, Aberdeen, UK), based on the type and quantity of each ingredient consumed and published food composition tables (FSA, McCance & Widdowson, 2002). Faecal samples were collected on average twice each week. In addition one 24 h collection from the final week of each week period was used for chemical analysis of digestibility.

Chemical analysis of diet composition and digestibility

Diets were analyzed for total gross energy, resistant starch and insoluble and soluble non-starch polysaccharides (Table S2). Estimation of resistant starch content of diets and 24h faecal sample collections was as described by Englyst *et al.* (1992) while non-starch polysaccharides were quantified as described by Englyst & Cummings (1988).

DNA extraction from faecal samples

Faecal samples were kept at 4°C and processed within 5 hours of collection. This short period of storage is not expected to influence molecular estimation of microbial community composition (Lauber *et al.*, 2010). Each sample was mixed and 5 g dispersed (3500 rpm for 1 min using a Dispomix Drive (Media Tools, Switzerland)) in 10 ml sterile PBS buffer before aliquoting. One aliquot was used immediately for DNA extraction using the FastDNA Spin for soil kit following manufacturer's (Qbiogene) instructions.

Phylogenetic analysis of sequences derived from 16S rRNA clone libraries

PCR amplification of 16S rRNA genes from the extracted DNA involved initial denaturation at 94°C for 5 min; 20 cycles of denaturation (30 s at 95°C), annealing (30s at 52°C), extension at 72°C for 2 min with a final cycle at 72°C for 8 min. Taq1 polymerase from Promega was used at 0.025 units/ μl in the presence of 2.5 mM $MgCl_2$. The forward primer comprised a 4:1:1:1:1 mixture of 7f, 27fChl, 27fBor, 27fBif and 27fAto and the reverse primer was 1510r (Table S3). PCR products were cleaned using the Wizard PCR product purification kit (Promega, Southampton, UK) and were then cloned and sequenced as described previously (Lawley *et al.*, 2009). The sequences (spanning variable regions V2-V5) were aligned using the SILVA-derived reference alignment in the mothur software package (Schloss *et al.*, 2009) and were tested for the presence of chimeras by Mallard (Ashelford *et al.*, 2006), Pintail (Ashelford *et al.*, 2005) and BLAST (Johnson *et al.*, 2008). After removal of chimeric sequences the alignment was subjected to extensive manual curation using the editor function in the ARB package (Ludwig *et al.*, 2004). Using the curated alignment a distance matrix, with Felsenstein correction, was created using ARB. This matrix was then used as an input for DOTUR (Schloss & Handelsman, 2005) using a 98% identity cut-off under the default furthest-neighbour setting. Sequences with > 98% phylogenetic similarity were regarded as belonging to the same phylotype. This generated

320 phylotypes (Table S4), which were assigned an identity from the phylum to genus level by the RDP Classifier (Cole *et al.*, 2009) and to the closest match in the NCBI database using MegaBLAST (Johnson *et al.*, 2008). Rarefaction analysis, Shannon and Simpson diversity index calculations, community structure comparisons with ∫-Libshuff, parsimony and weighted and unweighted versions of UniFrac, Jaccard and Yue & Clayton theta tree clustering analysis were all performed using the mothur software package (Schloss *et al.*, 2009).

The final 16S rRNA clone library dataset from six volunteers (v16, v19, v20, v22, v23, v24) contained 5915 sequences (median length of 678 bases) (GenBank accession numbers GU238434-GU244348). These sequences were from one faecal sample from each of the four diets per individual, except for volunteer v16 when two samples were analyzed from the M and NSP diets (see Table S4). An additional 768 sequences were subsequently obtained from the RS and NSP diets only of v14 and v25 (Table S5) (GenBank accession numbers HM191774-HM192541).

qPCR and DGGE analysis of 16S rRNA genes

qPCR was performed on all available samples from all 14 volunteers. Primer sequences and conditions are reported in Table S3, based largely on Ramirez-Farias *et al.* (2009). Primers for *Oscillibacter*-related bacteria were designed in this study. For DGGE, bacterial DNA was amplified by PCR using primers for the hyper-variable V3 region of the 16S gene (Table S3). Reaction mixtures contained 1.5 mM $MgCl_2$ and 0.025 units Taq1 polymerase/ μl. Products were separated in an 8% acrylamide denaturing gradient gel containing urea and formamide (35% to 60% gradient) at 80V for 16h at 60°C and visualized by staining with SybrGold dye (Invitrogen).

Statistical analysis

For phylotypes with large abundance (more than 1% and found in 5 or 6 out of 6 volunteers, which was the case for 8 out of 320 phylotypes) the Sanger sequences (clone counts) were analysed as Hierarchical Generalised Linear Models using a binomial model with logistic link and estimated over-dispersion. This was done for one phylotype at a time, with the total number of clones per sample regarded as the total count. Volunteer and diet were regarded as random (normally distributed) and fixed effects, respectively. Similarities between faecal samples were displayed graphically by means of Principal Coordinate analysis (PCO), where the Canberra distance matrix was applied to the numbers of clones assigned to each phylotype (based on all 320 phylotypes) for each faecal sample. PCO was also used to obtain a graphical representation of the phylogenetic distances (obtained from the DOTUR software package) between the 320 phylotypes.

The effect of diet was tested by means of Analysis of Variance (ANOVA) with Volunteer regarded as random and diet regarded as fixed effect. When the effect of diet was significant, diet means were compared by post-hoc t-test. Data containing many zeros (i.e. below limit of detection) were analyzed by Friedman's non-parametric ANOVA instead. For all analyses, the effect of order was included initially as a fixed effect but it and its interaction with diet were found not to be significant and was removed from subsequent analyses. All statistical analyses were performed by using Genstat 11th Edition Release 11.1 (VSN International Ltd, Hemel Hempstead, Herts., UK). Significance was set at $P < 0.05$.

Results

Study design

Fourteen overweight male volunteers completed this study (see Materials & Methods) (Table S1). Complete diets were provided daily resulting in precise control over all dietary intake throughout the 10 week study period (Table S2). Each volunteer received a weight maintenance (M) control diet for the first week, followed by three weeks each of diets high in Novelose type III RS (RS diet), high in NSP (NSP diet, containing added wheat bran) and a weight loss diet reduced in carbohydrate but high in protein (WL diet). The first three diets were provided at energy maintenance and were closely matched for total carbohydrate, protein and fat content; the order of the RS and NSP diets was reversed for half of the volunteers (Fig. S1, Table S2). Faecal samples were processed as soon as possible after collection, without prior freezing, for the extraction of nucleic acids (see Materials and Methods).

Dominant bacterial phylotypes within the intestinal bacterial community

16S rRNA profiles from all available faecal samples were analysed first using DGGE, which revealed that profiles were consistent over time within an individual for a given diet (Fig. S2). A switch between the RS and NSP diets, however, altered the profile substantially, indicating a shift in bacterial community composition. Sequences of amplified 16S rRNA genes were analysed for six of the 14 volunteers (three from each diet order) using a sample from the final week of each dietary regime. This yielded 5915 non-chimeric sequences (averaging 227 sequences per sample). Phylogenetic analysis using a 98% sequence identity cut-off revealed 320 phylotypes across all samples (Table S4). The proportion of cultured strains increased with increasing abundance of phylotypes, with all phylotypes present at >2% of the total being identified with cultured species (Fig. 1). None of the 320 phylotypes was detected in every sample, but 32 phylotypes were present in all six volunteers accounting for 47.1% of all sequences, while 77 phylotypes occurred in at least four of the six individuals (Fig. 2, Fig. S3). Fig. 2 suggests a bimodal frequency distribution for phylotype occurrence, indicating that there may be a group of widespread and highly abundant phylotypes within the human gut microbiota (Tap *et al.*, 2009). The most abundant phylotype was *Eubacterium rectale* although three phylotypes corresponding to three divergent strains of *Faecalibacterium prausnitzii* together comprised 8.0% of total sequences (Table 1).

Overall community structure was analysed for each sample using the mothur software package (Schloss *et al.*, 2009). ∫-Libshuff, parsimony and UniFrac (weighted and unweighted) analysis revealed a high degree of variation between individuals and showed that each sample was significantly different from all others. Similarity in community membership and structure was estimated using the Jaccard and the Yue & Clayton theta similarity coefficients (Yue & Clayton, 2005) (Fig, S4). These results and those from principal co-ordinate analysis (Fig. 3) indicate that samples predominantly clustered by volunteer, suggesting that responses to dietary change are influenced by the initial species composition of the individual's colonic microbiota. Rarefaction analysis revealed that the observed species richness was similar for all four diets (Fig. S5). Simpson (1/D) and Shannon diversity indices were slightly lower on the RS and NSP diets compared with the M and WL diets, but did not differ significantly (Fig. S5; see also Table S4 for individual values).

Response of the colonic microbial community to dietary change

At the phylum level there was no significant effect of diet upon the proportions of Bacteroidetes (mean 21.5%), Firmicutes (mean 70.6%) Actinobacteria (mean 4.9%) or

Proteobacteria (mean 3.0%) within the faecal microbiota. At finer taxonomic levels however, two individual phylotypes, *E. rectale* and *Ruminococcus bromii*, showed increased proportions on the RS diet while *Collinsella aerofaciens* showed decreased proportions on the WL diet ($P < 0.001$) (Table 1). Since the sequence data also suggested that groups of related phylotypes might be affected by diet we decided to employ quantitative real-time PCR (qPCR) for further analysis.

PCR primer sets (Table S3) were used to target groups for which sequence analysis indicated a response to the RS diet (Rum, Ros, Osc), or for which previous *in vitro* evidence has demonstrated the ability to utilise starch (Bac, Bif, Fprau) (Table 2). Methanogens were also monitored because of their potential impact on the polysaccharide-utilizing community (Robert & Bernalier-Donadille 2003). All available time-points were analyzed. Mean data for the targeted groups are shown in Table 2 and complete timecourses for all 14 subjects are shown in Fig. 4 for those bacterial groups that showed responses to the RS diet. The Rum group includes relatives of *R. bromii, R. flavefaciens* and *R. albus* that belong to the *Ruminococcaceae*, and will be referred to here as 'R-ruminococci'. There was a significant increase of around 4.5 fold ($p < 0.001$) in this group on the RS diet, compared to the NSP diet (Table 2, Fig. 4). A new primer set was designed to recognize another group of *Ruminococcaceae*, related to *Oscillibacter valericigenes* (Iino *et al.*, 2007) that has not been cultured from the human gut. This group increased significantly on average on the RS and WL relative to the M and NSP diets ($p<0.001$) (Table 2, Fig. 4). A previously reported primer set (Mackie *et al.*, 2003) targeting relatives of this group from the rumen was also used here, but detected <0.1% of human faecal bacteria (results not shown). Populations of a third abundant group of *Ruminococcaceae*, *F. prausnitzii*, showed no significant response to changes of diet (Table 2).

Firmicutes bacteria related to *Roseburia* and *E. rectale* also increased significantly ($P<0.048$) on the RS diet, and decreased (approximately 3-fold compared to RS) ($P<0.029$) on the reduced carbohydrate WL diet. (Ros, Table 2). Individual differences were evident for all groups shown in Fig. 4. Notably, *Bifidobacterium* spp. did not show a significant mean response to RS, but their population increased markedly on RS in one volunteer (v23) (Figs. 4, 5). Bifidobacteria are often poorly represented in 16S rRNA clone libraries (Eckburg *et al.*, 2005, Hold *et al.*, 2002) and this was also the case in our analysis in spite of using a broad bacterial primer set reported to include this group (Frank *et al.*, 2008). There was no significant change in the % *Bacteroides* in response to diet (Table 2).

Methanogenic archaea were detected by qPCR in >90% of samples from nine volunteers, but were detected in fewer than 10% of samples from v11, 14, 17, 24 and 25. This group showed no significant effect of diet (Table 2).

Digestibility of resistant starch and NSP

Whole tract digestibility of resistant starch and NSP was estimated from chemical analysis of the diet and of 24 h faecal collections. For 12 of the 14 volunteers less than 4% of ingested RS was recovered in faecal samples for all four diets, reflecting almost complete microbial fermentation. For two individuals (v14 and v25), however, faecal RS recovery was 69% and 65% respectively (31% and 35% digestibility) on the RS diet (Fig. 6). These two individuals both harboured low R-ruminococci numbers as assessed by pPCR, although one of them showed an increase in the *Roseburia* and *E. rectale* group on the RS diet (Figs. 4, 5). Additional clone library analyses were subsequently performed on the final samples from the RS and NSP dietary periods for these two individuals. No R-ruminococci were detected while 36% and 46.5% of clones belonged to the *Bacteroidetes* among the 381 and 387 clones analysed for v14 and v25 respectively (Table S5).

Soluble NSP digestibility averaged 90% and was slightly lower ($p<0.05$) for RS and WL diets (88%) than for the maintenance diet (92%). Insoluble NSP digestibility averaged 66% and showed no difference between diets (mean range 58 to 71%).

Discussion

This is the first study to combine precise control and monitoring of human dietary intake and digestibility with detailed analysis of changes in the faecal microbiota at the level of bacterial phylotypes. As a result it has been possible to reveal the interplay between diet and inter-individual differences that influence the human colonic community. Analysis of amplified 16S rRNA sequences suggested that samples from six overweight male volunteers clustered more strongly by individual than by diet. This is in line with previously reported evidence of inter-individual variation in the faecal microbiota (Ley *et al.*, 2006; Franks *et al.*, 1998). On the other hand, diet composition had very substantial effects on specific groups of bacteria that were detected both through clone libraries and extensive qPCR analysis on 279 faecal samples. The time-courses show that most diet-driven changes occurred rapidly, being detectable within three to four days, and were reversed equally rapidly. These kinetics appear consistent with immediate effects of dietary residue upon relative bacterial growth rates in the colon that are subsequently reflected in faecal samples as colonic contents turnover, assuming mean transit colonic times of around 60 h (Stephen *et al.*, 1987).

Firmicutes bacteria related to *R. bromii* (R-ruminococci) and *E. rectale* were commonly stimulated by the RS diet. In most individuals qPCR analysis revealed a surge in the population of R-ruminococci with values exceeding 25% of total bacteria in some samples. R-ruminococci were the only group of human gut bacteria previously found to be preferentially associated with particulate material in human faecal samples (Walker *et al.*, 2008), suggesting that they play an important role in the breakdown of particulate substrates. *R. bromii* isolates show amylolytic activity (Salyers *et al.*, 1977) but our results suggest that many related, but uncultured, bacteria may also possess this activity. One previous study, using non-quantitative DGGE analysis, reported that *R. bromii*-related bacteria were prominent in faecal samples from humans on a diet high in resistant starch and non-starch polysaccharides (Abell *et al.*, 2008). The *E. rectale* and *Roseburia* group is also known to contain amylolytic species (Ramsay *et al.*, 2006) but has not previously been shown to respond to RS in vivo. An earlier study failed to detect an increase in specific *Eubacterium* spp. in human subjects on a diet high in RS type III but, importantly, detection methods were not available for *E. rectale* (Schwiertz *et al.*, 2002). In the present work we also detected a dietary response for an uncultured group of *Ruminococcaceae* related to *Oscillibacter*. It is not known whether these bacteria are starch degraders, and their increase both on the RS and WL diets suggests that other factors must be involved in their response to diet.

Early studies indicated a broad distribution of amylase activity among cultured human gut bacteria with > 50% of strains, including many *Bacteroides*, able to grow on amylose or amylopectin starch (Salyers *et al.*, 1977). The potential role of *Bifidobacterium* spp. has been emphasised particularly in the degradation of high amylose resistant starch (Wang *et al.*, 1999; Macfarlane & Englyst, 1986). In the present study bifidobacterial numbers showed a strong response to RS only in one individual (v23), while there was no evidence for a response of the *Bacteroides* group to RS. *Bacteroides* spp. may perhaps be better adapted to the utilization of solubilized starch molecules (Flint *et al.*, 2008). The present findings *in vivo* however correspond better with more recent work *in vitro* that identified *R. bromii*, *E. rectale* and *Bifidobacterium* spp. as the major species in human faeces that colonise starch particles (Leitch *et al.*, 2007) and that utilise ^{13}C-labelled starch (Kovatcheva-Datchary *et al.*, 2009).

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Different groups of amylolytic bacteria differ markedly in their metabolic products and potential host interactions. *Bifidobacterium* spp., which produce lactate and acetate, are widely used as probiotics and as targets for prebiosis (Furrie *et al.*, 2005). Members of the *E. rectale* group are flagellated bacteria that are major producers of butyrate in the large intestine, and may therefore contribute to the butyrogenic effect of resistant starch (Aminov *et al.*, 2006; Duncan *et al.*, 2007; Louis *et al.*, 2010). *R. bromii* is a producer of acetate, ethanol and hydrogen that is likely to contribute to gas production, but otherwise little is currently known about the impact of this group upon the host. It will be important in future to establish whether different types of resistant starch select for different groups of amylolytic bacteria in view of their differing effects on fermentation and host responses (Le Leu *et al.*, 2009).

Starch is considered to show high digestibility across the whole GI tract, with most resistant starches being completely fermented in the large intestine (Bird *et al.*, 2000). Remarkably, however, significant amounts of starch survived fermentation to be recovered in the faeces in two of the fourteen individuals studied here. This difference in fermentation cannot be ascribed to dietary intake, which was standardised for all 14 subjects, and seems likely to lie with the strain composition of each individual's colonic microbiota. These two individuals showed very low numbers of R-ruminococci, and the relationship between community composition and starch fermentation will clearly warrant further investigation. Although these two individuals were also non-methanogenic, no simple relationship was evident between methanogens and R-ruminococci. Methanogen populations are known to vary between individuals and are influenced by a variety of factors (Florin *et al.*, 2000).

In contrast to these responses to RS, there was little evidence that the high NSP diet resulted in major alterations in the composition of the faecal microbiota. In part, however, this may reflect the fact that a smaller increase was achieved in NSP intake (1.5 fold) than with RS intake (4.8 fold) when compared with the maintenance diet. It is possible that larger changes in specific NSP components would affect the populations of specific groups of colonic bacteria, as was observed with RS. Significant decreases were observed for *C. aerofaciens* and for the *E. rectale* group on the WL diet. The WL changes do not show a simple relationship with RS and NSP intakes, and it is possible that the increased dietary protein content of this diet might play a role in altering microbiota composition.

The distribution of major bacterial phyla observed here did not depart dramatically from that reported in non-obese subjects (Eckburg *et al.*, 2005; Walker *et al.*, 2008). The data that we obtained by qPCR and clone library analysis are consistent with recent reports on obese subjects using FISH microscopy (Duncan *et al.*, 2007, 2008; Schwiertz *et al.*, 2010) or 16S rRNA sequencing (Zhang *et al.*, 2008) although lower % *Bacteroides* have been reported in another study (Ley *et al.*, 2006). More subtle differences may occur between the gut microbiota of obese and non-obese individuals at the species level, and indeed the dietary responses reported here make this likely. Nevertheless five of the 10 most abundant phylotypes identified in the present study group were also among the 10 most abundant phylotypes described by Tap *et al* (2009) in non-obese volunteers.

A high proportion of the most abundant 16S rRNA phylotypes that we detected here corresponded to cultured bacteria, with 66% of the 50 phylotypes that accounted for >0.5% of sequences having close cultured relatives. This suggests that the limited coverage of the human gut microbiota through cultivation may be due to insufficient anaerobic isolation work, rather than to intrinsic non-culturability of human colonic bacteria. Four of the five most abundant phylotypes detected here (*E. rectale, F. prausnitzii, C. aerofaciens* and *B. vulgatus*) corresponded with the most abundant species reported by anaerobic cultivation in Japanese–Hawaiians, North American Caucasians and polyp patients (Moore *et al.*, 1995).

Overall, however, 33.4% of phylotypes showed <98% identity with cultured bacteria in the present study, reflecting the relatively poor coverage of the less abundant bacterial groups by cultured strains. Many abundant phylotypes were found to be widespread, with 32 of the 320 phylotypes detected being present in all six individuals surveyed and accounting for almost half (47%) of all sequences.

In conclusion, we find that increased intake of resistant starch, an important non-digestible carbohydrate in the human diet, can substantially alter the species composition of the colonic microbiota. Such responses are likely to occur also with other fermentable ND dietary components. It follows that the colonic microbial community must typically be in a state of continuous change over time, driven by short–term changes in dietary intake. Thus only the most successful and versatile organisms will be found commonly among the dominant microbiota at different sampling times and in different individuals. In addition, however, our evidence demonstrates that the bacterial strain composition of the colonic microbiota is subject to inter-individual variation. Furthermore we show that this can be associated with profound inter-individual differences in the response of the microbial community to dietary change, and in microbial fermentation of dietary substrates in the colon. This suggests that dietary advice on the consumption of ND carbohydrates might need to be personalized in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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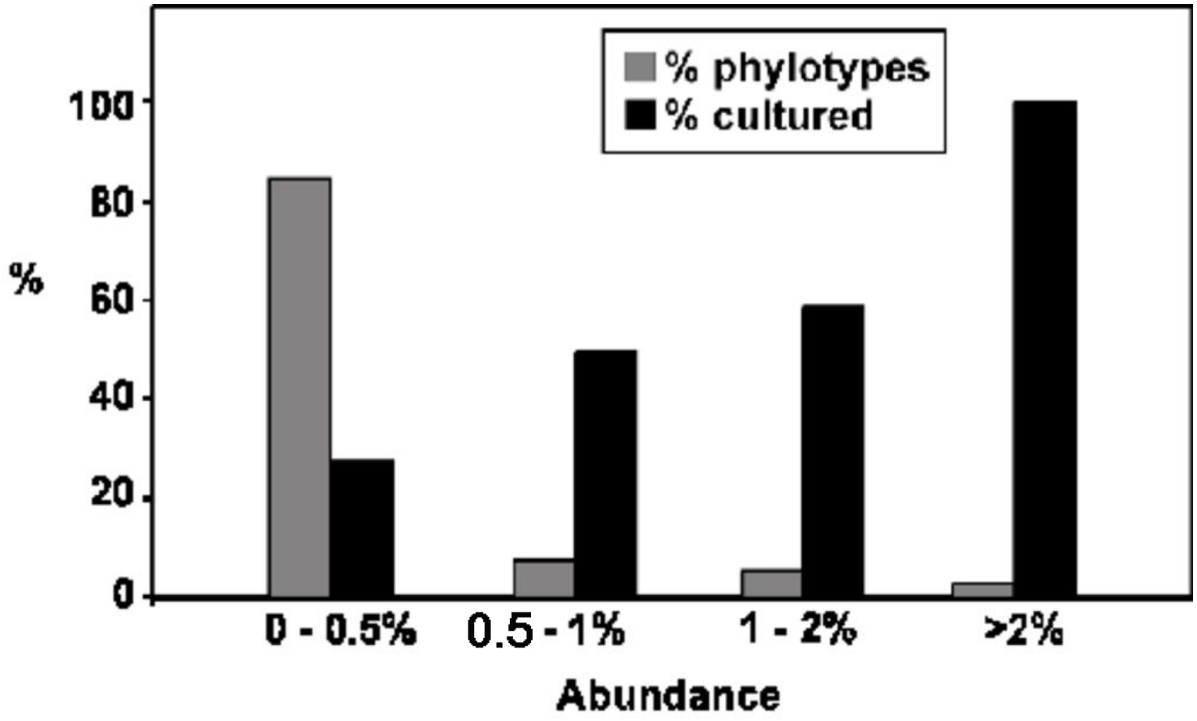

Figure 1. Culturability of 16S rRNA phylotypes in relation to their abundance

5915 16S rRNA sequences obtained from faecal samples of six volunteers (for all four diets) were classified into 320 phylotypes (defined at >98% sequence identity) (Table S4). The % of phylotypes showing >98% sequence identity to a cultured bacterium is seen to increase with increasing phylotype abundance. The phylotype frequency distribution is shown as a percentage; actual numbers of phylotypes were 9 (>2% of all sequences), 17 (>1%<2%), 24 (>0.5%<1%), and 270 (<0.5%) (total 320). Data refer to volunteers 16, 20, 22 (diet order RS-NSP) and 19, 23, 24 (diet order NSP-RS).

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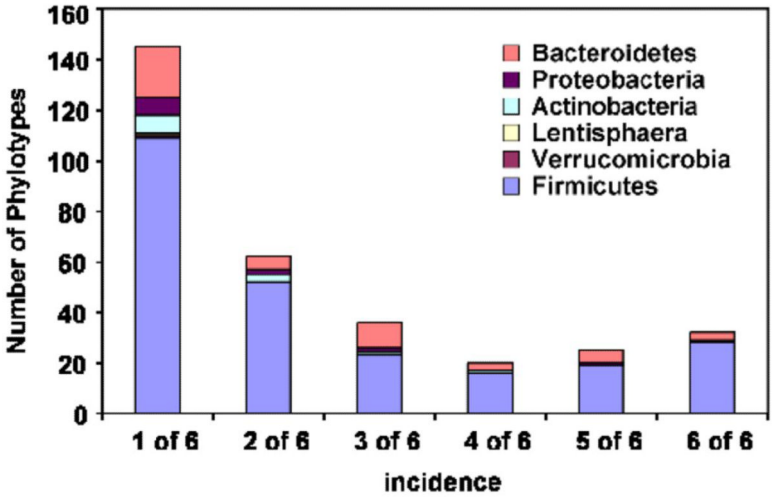

Figure 2. Incidence of phylotypes in different individuals

The distribution of all 320 16S rRNA phylotypes was determined across the six volunteers (see Fig. 1, Table S4); the numbers of phylotypes found in all six individuals (32) and in five (25), four (20), three (36), two (62) or one (145) of the six are shown here according to bacterial phylum.

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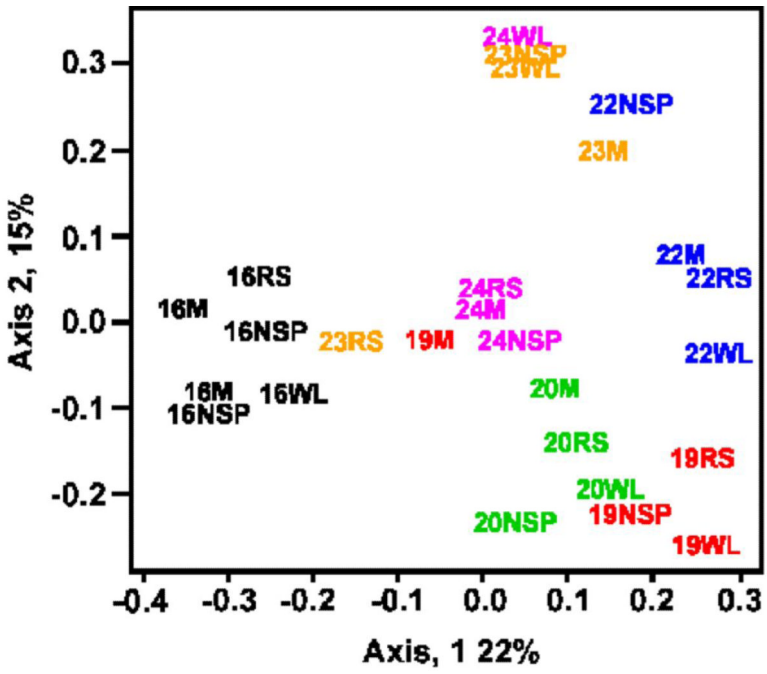

Figure 3. Impact of diet and individual variation upon faecal microbiota composition

A principal coordinates analysis (using Canberra distance matrix) based on 16S rRNA clone libraries from 26 faecal samples (obtained from six donors under four dietary conditions) (Table S4). Colour code is based on donor (v16, v19, v29, v22, v23, v24). Diets are indicated as:- M-maintenance, NSP-non-starch polysaccharide, RS-resistant starch, WL-weight loss. Two samples were analysed for v16 from the M and NSP diets.

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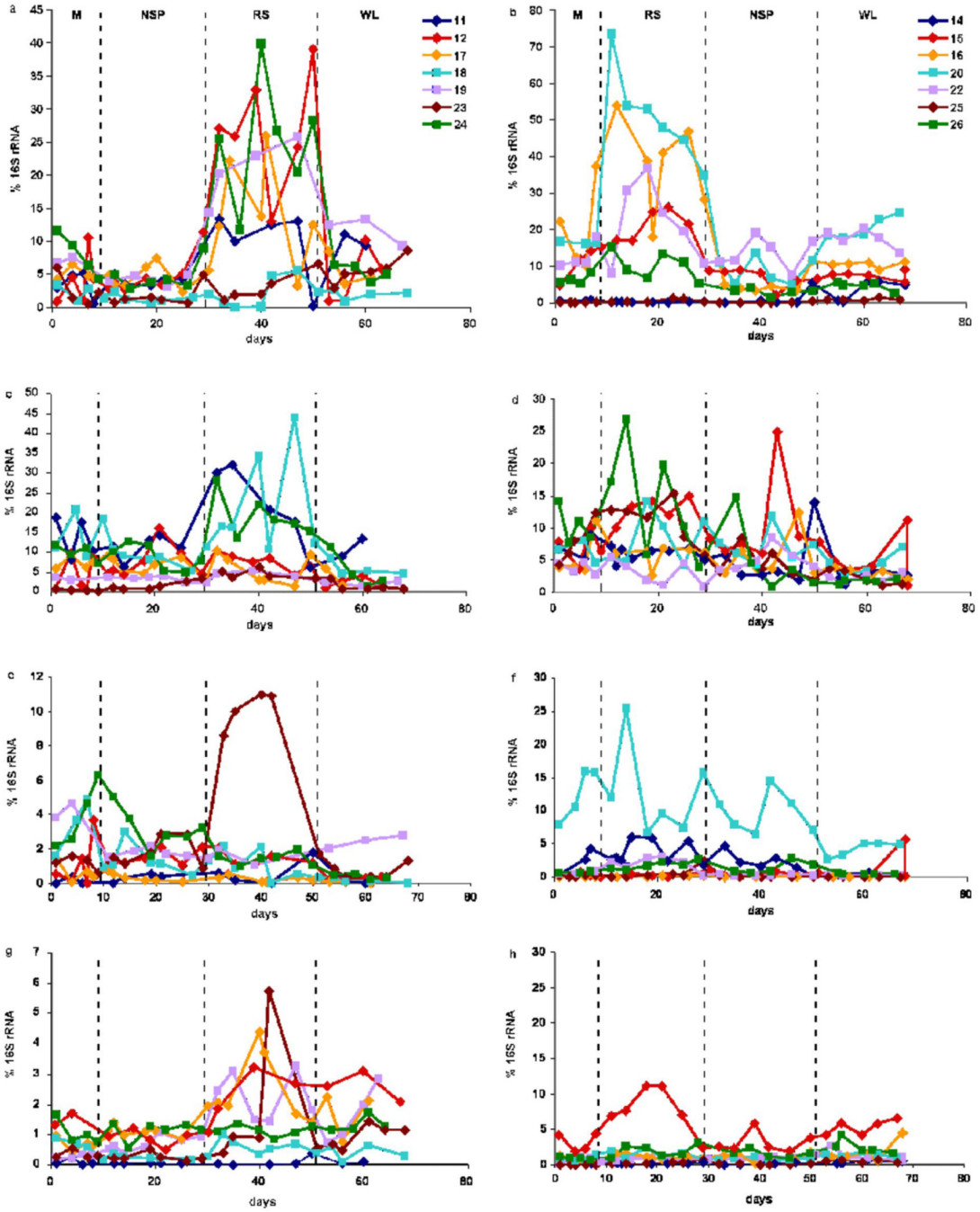

Figure 4. Diet-driven changes in four groups of human colonic bacteria detected by qPCR

Abundance for each targeted group is expressed as a percentage of the signal obtained with a general bacterial primer set (see Table S3 for primers and conditions used). All available time-points are shown for the 14 volunteers: left-hand panels show the diet order M-NSP-RS-WL and right hand panels M-RS-NSP-WL a), b) R-ruminococci (relatives of *R. bromii*); c), d) Relatives of *E. rectale* and *Roseburia* spp.; e), f) *Bifidobacterium* spp.; g), h) Relatives of *Oscillibacter valericigenes*.

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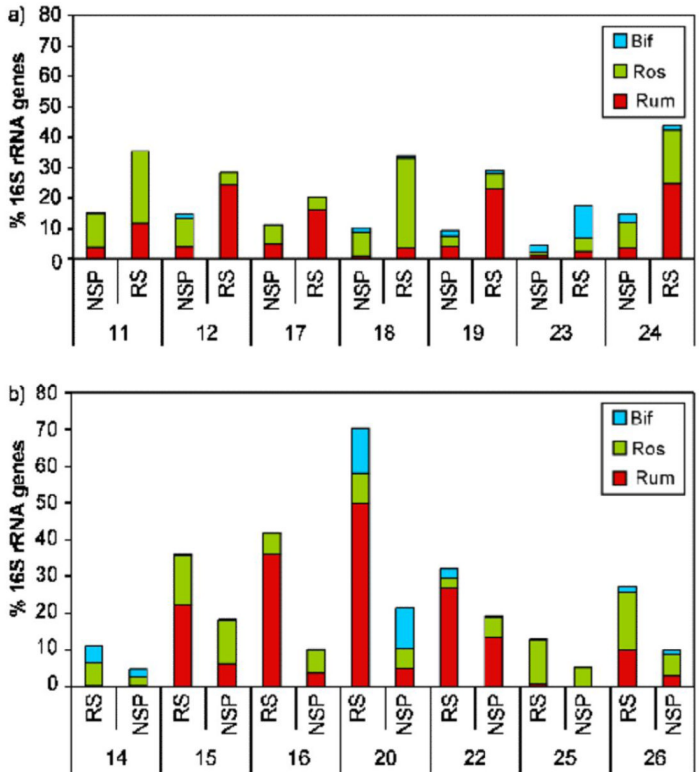

Fig. 5. Populations of three groups of potentially amylolytic bacteria on the RS (high resistant starch) and NSP (low resistant starch) diets

Populations, estimated by qPCR, are shown for R-ruminococci, *E. rectale/Roseburia* spp. and *Bifidobacterium* spp. for all 14 volunteers, according to diet order. Data are the mean values for each volunteer during the second and third week of the RS and NSP diets (overall means are given in Table 2).

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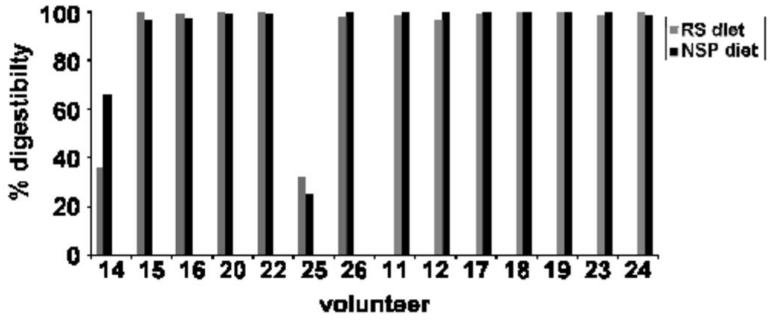

Figure 6. Starch digestibilities

The whole tract % digestibility of resistant starch was determined after estimating resistant starch present in the supplied diet and in 24 h faecal samples (see Materials and Methods). Results are shown only for the NSP and RS diet periods. The markedly reduced resistant starch digestibilities in v14 and v25 correspond with low R-ruminococcal numbers in these individuals (see text and Table S5).

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Table 1

Impact of diet upon the ten most abundant 16S rRNA phylotypes detected in six overweight male volunteers

			Mean % clones				
Phylotype, species[+]	**Phylum**[*]	**% total clones**	**Diets: M**	**NSP**	**RS**	**WL**	**P**[**]
Eubacterium rectale	*F (L)*	4.43	2.9[a] [**]	4.1[a]	8.0[b]	1.7[a]	< 0.001
Collinsella aerofaciens	*A*	3.67	4.3[a]	3.4[a]	5.2[a]	0.6[b]	0.032
Clostridium clostridioforme	*F (L)*	3.83	4.8	2.6	2.5	3.1	0.217
Bacteroides vulgatus	*B*	3.21	2.6	4.1	1.3	4.9	0.057
Faecalibacterium prausnitzii L2-6	*F (R)*	2.96	2.5	3.4	1.6	3.8	0.224
F. prausnitzii A2-165	*F (R)*	2.55	1.8	2.8	2.9	3.4	0.338
F. prausnitzii M21/2	*F (R)*	2.47	2.1	1.8	2.2	3.1	0.771
Anaerostipes coli[#] SSC/2	*F (L)*	2.25	3.0	2.5	1.1	1.8	0.091
Ruminococcus. bromii	*F (R)*	2.11	1.5[a]	0.4[a]	5.0[b]	1.5[a]	<0.001
Eubacterium hallii	*F (L)*	2.00	2.4	2.5	1.2	1.4	0.251

[+] Detected in all 6 volunteers, except for *R. bromii* (4/6 volunteers)

[*] F, Firmicutes (L, *Lachnospiraceae*; R, *Ruminococcaceae*); B, Bacteroidetes; A, Actinobacteria

[**] Within a row values not sharing superscripts differ significantly (P < 0.05). Clone counts were analysed with Hierarchical Generalised Linear Model with Volunteer as random and Diet as fixed effect.

[#] New species name soon to be formally proposed (Allen-Vercoe *et al.*, in preparation)

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Table 2

Populations estimated by qPCR of bacteria and methanogenic archaea expressed as % relative to total bacteria (Bac= *Bacteroides/ Prevotella*, Ros= *E. rectale/ Roseburia* spp, Fprau= *F. prausnitzii*, Rum= 'R-ruminococci', Osc= *Oscillibacter*-relatives, Bif= *Bifidobacterium* spp., Met= methanogens). Primer sequences and amplification conditions are shown in Table S3. One mean value is shown per diet for the 14 volunteers. For the M diet, means are for all samples from the one week of this diet, whilst for the NSP, RS and WL diets means are for all samples from the last two weeks on each diet.

	Bacterial group (%) :-					
	Diet: M	**NSP**	**RS**	**WL**	**SED**	**P diet***
Bac	27.8	25.7	20.2	24.0	3.58	0.387
Ros	7.3^{a} *	6.5^{a}	10.1^{b}	3.3^{c}	1.381	<0.001
Fprau	11.2	14.4	12.1	12.5	1.622	0.278
Rum	6.5^{a}	3.8^{a}	17.0^{b}	7.5^{a}	2.253	<0.001
Osc	0.74^{a}	0.77^{a}	2.0^{b}	1.6^{b}	0.34	<0.001
Bif	1.9	1.8	2.4	0.8	0.632	0.059
Met	0.080	0.034	0.124	0.123	0.042	0.155**

*Significance based on Analysis of Variance (ANOVA). Within each row, values not sharing superscripts differ significantly (P < 0.05, post-hoc t-test).

**Friedman non-parametric ANOVA.