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Reduced Dietary Intake of Carbohydrates by Obese Subjects Results in Decreased Concentrations of Butyrate and Butyrate-Producing Bacteria in Feces[▽]

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Weight loss diets for humans that are based on a high intake of protein but low intake of fermentable carbohydrate may alter microbial activity and bacterial populations in the large intestine and thus impact on gut health. In this study, 19 healthy, obese (body mass index range, 30 to 42) volunteers were given in succession three different diets: maintenance (M) for 3 days (399 g carbohydrate/day) and then high protein/medium (164 g/day) carbohydrate (HPMC) and high protein/low (24 g/day) carbohydrate (HPLC) each for 4 weeks. Stool samples were collected at the end of each dietary regimen. Total fecal short-chain fatty acids were 114 mM, 74 mM, and 56 mM ($P < 0.001$) for M, HPMC, and HPLC diets, respectively, and there was a disproportionate reduction in fecal butyrate (18 mM, 9 mM, and 4 mM, respectively; $P < 0.001$) with decreasing carbohydrate. Major groups of fecal bacteria were monitored using nine 16S rRNA-targeted fluorescence in situ hybridization probes, relative to counts obtained with the broad probe Eub338. No significant change was seen in the relative counts of the bacteroides (Bac303) (mean, 29.6%) or the clostridial cluster XIVa (Erec482, 23.3%), cluster IX (Prop853, 9.3%), or cluster IV (Fprau645, 11.6%; Rbro730 plus Rfla729, 9.3%) groups. In contrast, the *Roseburia* spp. and *Eubacterium rectale* subgroup of cluster XIVa (11%, 8%, and 3% for M, HPMC, and HPLC, respectively; $P < 0.001$) and bifidobacteria (4%, 2.1%, and 1.9%, respectively; $P = 0.026$) decreased as carbohydrate intake decreased. The abundance of butyrate-producing bacteria related to *Roseburia* spp. and *E. rectale* correlated well with the decline in fecal butyrate.

Low-carbohydrate diets in which carbohydrates are largely replaced by an increased proportion of dietary protein and/or fat have proved a popular weight loss strategy for humans (1, 11, 36). The potential health impacts associated with increased protein (34) or fat (27) intake have been controversial among nutritionists, but less attention has been paid to the consequences of low carbohydrate supply. It has been argued that a lower carbohydrate supply may be advantageous in ameliorating insulin insensitivity (9), although this may not occur with low-glycemic-index foods (21). Furthermore, in the context of overall dietary advice consideration also needs to be given to the role of carbohydrates in maintenance of gut health and function.

Dietary carbohydrates include structural polysaccharides and oligosaccharides of plant origin plus resistant starch (14, 40) that are not digested in the small intestine and, instead, enter the colon. Here they can be fermented by the microbiota of the large intestine and normally provide the main energy supply to support microbial growth in the colon. Microbial fermentation may release as much as 10% of the dietary energy, mainly in the form of short-chain fatty acids (SCFA) that also act as energy sources for host cells (46). For example, butyrate is the preferred energy source for the epithelial cells of the colon (29, 50). Furthermore, butyrate has been implicated in the prevention of colitis and colorectal cancer (16, 35, 44, 53, 61).

Reduced intake of fermentable dietary carbohydrate might be expected to impact on both the activity and the abundance of the different bacterial groups that populate the large intestine (28, 37). The aim of the present study was therefore to investigate the effect of reduced carbohydrate intake upon bacterial populations and metabolites detected in fecal samples. Quantification of bacterial groups involved in particular metabolic roles is now feasible following development of specific 16S rRNA-targeted probes for many human colonic bacterial groups (25, 39, 48, 60). A panel of probes including those targeted to the most abundant groups of butyrate-producing bacteria found in human fecal samples (3, 6, 33, 60) was used to monitor the effect of a dietary shift from normal intakes of carbohydrate (399 g/day) to either moderate (164 g/day) or low (24 g/day) intakes as part of weight loss strategies in obese men. Significant relationships were established between dietary carbohydrate intake, the composition of the fecal microbiota, and fecal SCFA concentrations.

MATERIALS AND METHODS

Volunteer recruitment. Obese, but otherwise healthy, male volunteers ($n = 20$) were recruited for a 9-week dietary intervention study (A. M. Johnstone, G. Horgan, S. Murison, D. M. Bremner, and G. E. Lobley, submitted for publication). One subject left the study early for reasons not associated with the protocol. All collected samples were analyzed. The volunteers were aged 36.7 ± 2.3 years (mean ± standard error of the mean; range, 20 to 57 years) with a mean body mass index (kg/m^2) of 35.4 ± 0.9 (range, 30 to 42). Volunteers were selected

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TABLE 1. Dietary intake (g/day) indicating mean values for 7 days preceding fecal sample (3 days for maintenance diet)

Diet	Fat	Protein	Carbohydrate	Nonstarch polysaccharides	Starch
Maintenance	122.9	94.3	398.8	27.9	187.3
HPMC	74.3	127.2	163.6	11.7	95.3
HPLC	126.0	119.5	23.9	6.1	2.7
P value	<0.001	0.001	<0.001	<0.001	<0.001

based on absence of indices of metabolic syndrome and had no history of gastrointestinal problems. No antibiotics or drugs known to influence the fecal microbiota were taken during the course of the study. Ethical approval was granted by the Grampian Research Ethics Committee, and all volunteers provided informed, signed consent.

Experimental design. This study, conducted over a 9-week period, included three 3-day intakes at energy maintenance with two intervening main diet periods, at either low or moderate carbohydrate intake, each for 28 days with the order randomized between subjects. Fecal samples were collected on three occasions, after 3 days on the first maintenance period and during the last 2 days on each of the main diets. Food intakes were quantified by weight, with any refusals also weighed.

Experimental dietary regimen. The volunteers were weight stable (less than 2-kg change in recent months) on entry to the trial and were then offered an energy maintenance (M) diet (based on 1.6× resting metabolic rate) for 3 days. This diet comprised 13% protein, 52% carbohydrate, and 35% fat as calories. Subjects were then offered ad libitum two diets, which were either a high-protein, low-carbohydrate (HPLC; 30% protein, 4% carbohydrate, 66% fat as calories) diet or a high-protein, moderate-carbohydrate (HPMC; 30% protein, 35% carbohydrate, 35% fat) diet, each supplied for 4 weeks in a randomized crossover design. Between the two main diet periods and at the end of the study the subjects were given the maintenance diet for 3 days. All meals were of the same energy density (5.5 MJ/kg), and daily intakes were recorded by weight. Daily macronutrient intakes were calculated using the Windiet software program (Robert Gordon University, Aberdeen, United Kingdom), based on the type and quantity of each ingredient consumed and published food composition tables (24). Diet intake was analyzed (Johnstone et al., submitted) for maintenance, HPMC, and HPLC diets (Table 1).

Collection and preparation of fecal samples for analyses. The fresh fecal samples provided from each volunteer were maintained at 4°C prior to processing on the same day as collection. Each sample was mixed, and four 0.5-g samples were taken for further analyses, which were performed blind. These were stored at −20°C for metabolite analyses or directly processed for fluorescence in situ hybridization (FISH) analysis.

Enumeration of bacteria in fecal samples by FISH analysis. The fecal sample was mixed, and a subsample (0.5 g) was thoroughly mixed with 4.5 ml phosphate-buffered saline and fixed by mixing 1:3 in 4% (wt/vol) paraformaldehyde at 4°C for 16 h. Subsamples (0.8 ml) were stored at −20°C. FISH analysis was performed on the liquid phase (20, 31). Briefly, diluted cell suspensions were applied to gelatin-coated slides. Slides were then hybridized with 10 μl of the respective oligonucleotide probe (50 ng/μl stock solution) and washed. Cells were counted automatically using a DMRXA epifluorescence microscope (Leica) and image analysis software, except when the number of fluorescent cells was less than 10 per field of view, when the cells were counted manually. Total bacterial numbers were estimated with the Eub338 probe (2) while the other bacterial groups were assessed using a panel of nine probes (Table 2).

Fermentation product analysis. The SCFA content of the samples was determined by capillary gas chromatography following conversion to *t*-butyldimethylsilyl derivatives (52). The lower limit for reliable detection of each product was taken as 0.2 mM. Ammonia concentrations were analyzed in samples diluted (1:3) with sterile water and then reacted with sodium phenate and sodium hypochlorite, with the optical density of the indophenol blue produced determined at 625 nm (62).

Statistical analysis. The data analyzed were obtained from 19 volunteers, 14 of whom provided fecal samples for all three dietary periods. The remaining five volunteers provided samples for two periods only. As a result, data consisted of 18 samples each for the maintenance and HPLC diets and 16 samples for the HPMC diet. Data from the maintenance diet were from the 3-day period at the start of the study. SCFA concentrations and bacterial data, both as % and as log(bacterial count), were analyzed as one-way analysis of variance (ANOVA) with the subject as a random effect and diet as a fixed effect. The effect of order was initially included but was found to be not significant and so was excluded from subsequent analyses. When the effect of diet was significant, Tukey's test was used to compare diet means. Several of the samples had undetectable levels of the fermentation products lactate, formate, and succinate and numbers of lactobacilli. Consequently, these data did not meet the ANOVA requirements of normality and equality of variance and were therefore analyzed by Friedman's nonparametric ANOVA. The effects of carbohydrate intake and bacterial counts on fecal butyrate concentration were investigated using restricted maximum likelihood (REML), with subject as a random effect and carbohydrate intake or bacterial count as fixed effect. All statistical analyses were performed by using Genstat 8th Edition Release 8.1 (VSN International Ltd., Hemel Hempstead, Herts, United Kingdom). Significance was set at $P < 5\%$.

TABLE 2. Bacterial groups recognized by the 16S rRNA-targeted fluorescence in situ hybridization probes

Probe	Probe target	Reference for probe
Eub338	Domain *Bacteria*	2
Erec482	Clostridial clusters XIVa and XIVb	25
Bac303	*Bacteroides-Prevotella* group	42
Fprau645	*F. prausnitzii* group (subgroup of cluster IV)	57
Bif164	*Bifidobacterium* genus	38
Rbro730/Rfla729	*Ruminococcus bromii* and *Ruminococcus flavefaciens* (subgroup of cluster IV)	31
Rrec584	*Roseburia* and *Eubacterium* group (subgroup of cluster XIVa)	60
Lab158	*Lactobacillus-Enterococcus* group	30
Prop853	Clostridial cluster IX	60
Dsv698	Sulfate-reducing bacteria	43

RESULTS

Fecal samples were analyzed from subjects consuming a maintenance (also referred to here as high-carbohydrate) diet and from the same subjects towards the end of 4-week periods on an HPMC or HPLC diet (see Materials and Methods).

Changes in fecal metabolites. Total SCFA concentrations were lower during consumption of the HPMC and HPLC diets than during the maintenance period (74 and 56 versus 114 mM, respectively; $P < 0.001$). This was also the case for acetate, propionate, and valerate concentrations ($P < 0.004$) but not for formate or isobutyrate (Table 3). Butyrate concentrations were also lower for the HPLC than for the HPMC diet ($P = 0.003$). For the predominant SCFA, while concentrations decreased by approximately 50% between maintenance and low-carbohydrate diets, a greater proportional decrease (75%) was observed for butyrate (Table 3). This resulted in changes in the proportions of individual to total SCFA. Thus, acetate proportion increased (0.57, 0.60, and 0.64, $P = 0.002$) as carbohydrate intake decreased while propionate proportion was unaltered (0.18 to 0.19, $P = 0.97$). In contrast, butyrate proportion decreased as carbohydrate supply was lowered (0.16, 0.11, and 0.07, $P < 0.001$). The relationship between carbohydrate intake and butyrate concentration was linear ($r = 0.76$, $P < 0.001$, REML analysis) (Fig. 1). Fecal ammonia also declined with decreased carbohydrate intake (Table 3).

Changes in fecal microbiota. Based on the broad bacterial probe (Eub338), bacterial numbers (log count per g feces) were greater on the maintenance diet than on the other two

TABLE 3. SCFA, lactate, and ammonia concentrations (mM)

Diet[a]	Formate	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Lactate	Succinate	Total SCFA	NH_3
M	0.56	65.09^{A}	20.28^{A}	2.27	17.67^{A}	2.13^{A}	3.14^{A}	1.23	1.27	113.6^{A}	51.67^{A}
HPMC	0.13	43.54^{B}	13.84^{B}	2.45	8.90^{B}	1.88^{AB}	2.04^{B}	0.13	0.79	73.7^{B}	42.98^{AB}
HPLC	0.27	35.50^{B}	10.81^{B}	1.91	4.36^{C}	1.34^{B}	1.38^{B}	0.00	0.63	56.2^{B}	33.03^{B}
SED[b]	0.44	4.11	1.86	0.29	1.40	0.27	0.35	0.53	0.50	7.17	5.34
P[b]	0.387[c]	<0.001	<0.001	0.105	<0.001	0.008	<0.001	0.009[c]	0.572[c]	<0.001	0.003

[a] $n = 18$ for M and HPLC diets; $n = 16$ for HPMC diet.
[b] Analyzed as one-way ANOVA with subject as random effect and diet as fixed effect. Standard error of the difference (SED) is based on 16 versus 18 observations. Diet means were compared by Tukey's test; different superscript capital letters within columns indicate $P < 0.05$.
[c] Analyzed with Friedman nonparametric ANOVA (no post hoc comparisons performed).

diets (10.71, 10.55, and 10.56, $P < 0.001$). The most abundant bacterial groups (Table 4) detected by the 16S rRNA-targeted FISH probes were the gram-negative *Cytophaga-Flavobacterium-Bacteroides* group (*Bacteroides* spp., detected by Bac303 probe; 29 to 30% of total bacteria detected with Eub338) and gram-positive bacteria belonging to the clostridial cluster XIVa (detected by Erec482; 21 to 24%) or the clostridial cluster IV (combined subgroups detected with the Fprau645 and Rum729/730 probes; 16 to 24%). Two other groups that made significant contributions were clostridial cluster IX bacteria (9 to 11%) and bifidobacteria (2 to 4%). Overall, the group probes used accounted for a large proportion of the total bacteria in the stool samples, 87.3%, 88.8%, and 82.1% from the maintenance, HPMC, and HPLC diets, respectively. The proportions observed for the maintenance diet are similar to those obtained previously in FISH surveys of fecal samples from healthy volunteers (25), although only one study has previously reported the abundance of the cluster IX group (60). Lower estimates were obtained for *Bacteroides* spp. by fluorescence-activated cell sorting (48), but the values here are comparable to those obtained by automated microscopy (25). Overall bacterial numbers obtained here with the Eub338 probe were slightly lower than reported by some previous studies that also employed DAPI (4′,6′-diamidino-2-phenylindole) detection (e.g., see references 25 and 31) where counts exceeded 10^{11}/g.

On the maintenance diet, bacteria detected with the Rrec584 probe, a subgroup of clostridial cluster XIVa, accounted, on average, for 11% (range, 6 to 21%) of the total Eub338 count (Table 4). This is slightly higher than reported recently for nonobese individuals with the same probe (3, 60). Bacteria targeted by the Rrec584 probe showed substantial decreases ($P < 0.001$) both in absolute numbers and as a proportion of total bacteria ($P < 0.003$; Table 4) as carbohydrate intake was lowered. This group of bacteria includes close relatives of *Roseburia intestinalis* and *Eubacterium rectale*, and all cultured representatives have butyrate as the main fermentation product from soluble sugars in pure culture (6, 18, 50). The decrease in numbers of bacteria within this group per gram feces paralleled the lowered fecal butyrate concentration (Fig. 2). This relationship was also observed within every individual (data not shown). Interestingly, relatives of *Faecalibacterium prausnitzii*, which have also been identified as a potentially important group of butyrate-producing bacteria (6), showed less of a response to reduced dietary carbohydrate and a weaker relationship ($r = 0.36$, $P = 0.005$ based on REML) with fecal butyrate concentrations.

All bacteria recognized by the Rrec584 probe (*Roseburia* spp. and *E. rectale* group) are also recognized by the Erec482 probe, because this group is part of the larger clostridial XIVa cluster. There was, however, no concomitant decrease in the clostridial cluster XIVa group (estimated with the Erec482 probe) on the lower-carbohydrate diets ($P = 0.17$), implying that other groups within the XIVa cluster increased ($P < 0.001$) as carbohydrate intake was reduced (Erec-Rrec, Table 4; XIVa-R, Fig. 3).

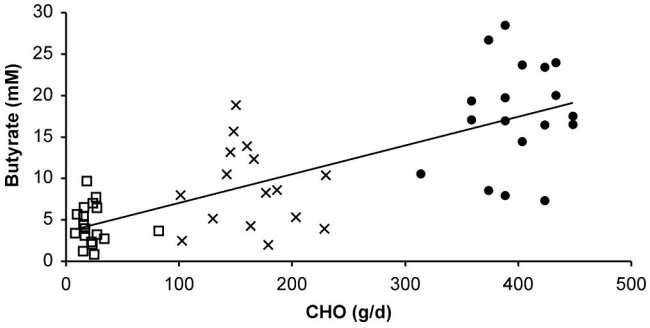

FIG. 1. Relationship between carbohydrate intake (average over 7 days preceding donation of stool sample for moderate- and low-carbohydrate diets; average of 3 days preceding stool sample for maintenance) and butyrate concentration in feces. ●, maintenance diet; ×, HPMC diet; □, HPLC diet. Correlation, 0.76 ($P < 0.001$, REML).

DISCUSSION

Total carbohydrate intake for the volunteers in this study decreased markedly from 399 g/day (maintenance) to 164 g/day (HPMC) and 24 g/day (HPLC). The predicted nonstarch polysaccharide (Englyst method) intakes were 28, 12, and 6 g/day ($P < 0.001$), respectively, while protein intake was lower for maintenance than for the other two diets (94, 127, and 120 g/day, $P = 0.003$) (Table 1). Carbohydrate content (26) and nonstarch polysaccharide intake were therefore lower than currently recommended in the United Kingdom (15) and in the United States (58).

In order to examine the impact of these dietary interventions upon the intestinal microbial community, a panel of targeted probes was employed that accounted for 82 to 89% of the total fecal bacteria detected, suggesting that most of the dominant species were covered. Previous studies with pure cultures provide an indication of the main substrates and metabolic prod-

TABLE 4. Abundance of bacterial groups (counts expressed as percentages of Eub338 counts) and total bacteria (log Eub338 count/g feces)

Diet[a]	Bac303	Fprau645	Rfla729 plus Rbro730	Bif164	Erec482	Prop853	Lab158	Dsv698	Rrec584	Erec-Rrec	Total log count/g feces
M	28.84	13.48	10.84	4.01^{A}	21.14	8.51	0.41	0.11	11.40^{A}	9.74^{A}	10.71^{A}
HPMC	29.96	11.39	10.00	2.09^{B}	24.40	10.76	0.11	0.13	7.79^{B}	16.61^{B}	10.55^{B}
HPLC	29.85	9.98	7.02	1.87^{B}	24.48	8.59	0.15	0.18	3.32^{C}	21.16^{C}	10.56^{B}
SED[b]	2.32	1.72	1.99	0.88	2.03	1.45	0.20	0.04	1.11	2.16	0.05
P[b]	0.774	0.089	0.102	0.026	0.173	0.254	0.353[c]	0.238	<0.001	<0.001	<0.001

[a] $n = 18$ observations for M and HPLC diets and 16 observations for HPMC diet.
[b] Analyzed as one-way ANOVA with subject as random effect and diet as fixed effect. Standard error of the difference (SED) is based on 16 versus 18 observations. Diet means were compared by Tukey's test; different superscript capital letters within columns indicate $P < 0.05$.
[c] Analyzed with Friedman nonparametric ANOVA (no post hoc comparisons were performed).

ucts for different phylogenetic groups of human colonic bacteria (6, 23, 54, 55), although it should be noted that metabolic cross-feeding is an important feature of the colonic microbial ecosystem (7, 19). The available evidence suggests substantial similarity in species composition between feces and colonic samples (22, 32). It should be stressed, however, that most of the SCFA produced within the colon are absorbed across the mucosa and more than 85% of butyrate formed by bacterial fermentation is metabolized by the colonic epithelial cells (8). Nevertheless, fecal concentrations can provide an important indicator of conditions within the distal colon, where the risk of colorectal cancer is highest. Invasive techniques have shown that butyrate flows in the cecal or portal veins simulate the pattern of production within the colon of pigs and humans (5).

The two bacterial groups previously reported to be most abundant in human fecal samples by FISH analysis (25), the bacteroides (*Cytophaga-Flavobacterium-Bacteroides*) group and the clostridial cluster XIVa (*Clostridium coccoides*) group, constituted approximately 29% and 22% of total bacteria, respectively, in these volunteers. Neither group changed significantly with a reduction in dietary carbohydrate. This study also demonstrated the abundance of the clostridial cluster IX group, detected with the Prop852 probe, at approximately 9% of total fecal bacteria, but again no significant effect of dietary carbohydrate was seen. On the other hand, relatives of *Roseburia* spp. and *E. rectale*, a subgroup of clostridial cluster XIVa, showed a significant and marked progressive decrease as a fraction of total bacterial cells with decreasing carbohydrate intake. It has been shown recently that this group includes many strains that are able to utilize dietary carbohydrates such as starch, xylan, and inulin for growth (18, 20, 51). An interesting corollary is that the remainder of the cluster XIVa responded positively to decreasing dietary carbohydrate. While it appears that bacteria of the *Roseburia* spp. and *E. rectale* group may be particularly dependent upon dietary carbohydrate supply in order to maintain their populations in the colon, there must be other groups within cluster XIVa that are relatively more successful at low carbohydrate intakes. *Roseburia* spp. and *E. rectale* comprised 11% of the total bacteria, which compares with a mean of 7% for 10 nonobese subjects studied previously using the same group probe (3). This dif-

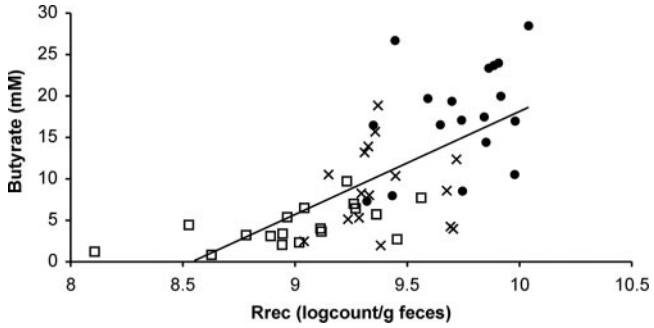

FIG. 2. Relationship between abundance of the *Roseburia* spp. and *E. rectale* group (detected using the Rrec584 probe) and butyrate concentration in feces. ●, maintenance diet; ×, HPMC diet; □, HPLC diet. Correlation, 0.68 ($P < 0.001$, REML).

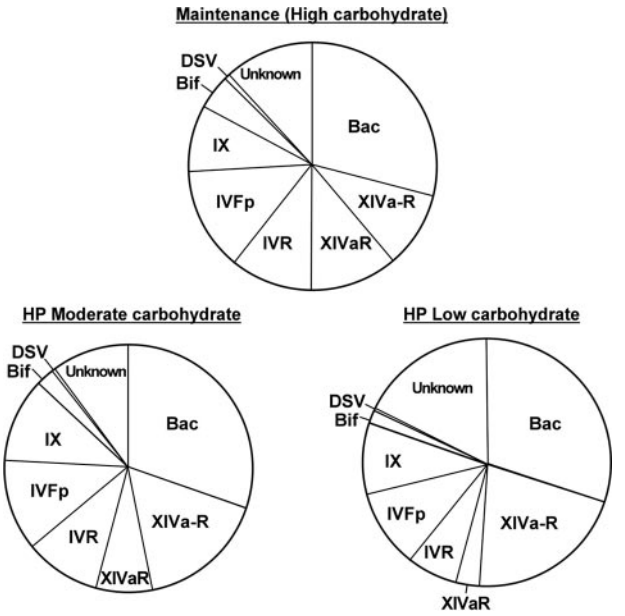

FIG. 3. Mean proportions of different bacterial groups in feces of human volunteers consuming maintenance, HPMC, or HPLC diets (assessed by FISH; also Table 4). The bacterial groups are represented as follows: Bac, *Bacteroides* spp. detected by Bac303; XIVaR, *Roseburia* spp. and *E. rectale*, detected by Rrec584; XIVa-R, clostridial cluster XIVa, detected by Erec482, minus those detected by Rrec584; IVR, cluster IV ruminococci detected by Rfla729 and Rbro730; IVFp, *F. prausnitzii* detected by Fprau645; IX, clostridial cluster IX bacteria detected by Prop853; Bif, *Bifidobacterium* spp. detected by Bif164; DSV, sulfate-reducing bacteria detected by Dsv698; unknown, bacteria detected by the broad Eub338 probe that were unaccounted for by the group probes used.

ference between studies may reflect either interindividual variation (the range was 6 to 21% for the current subjects) or, in light of the finding here, differences in the carbohydrate contents of the diets of free-living volunteers.

Two further groups were monitored that have been reported to include polysaccharide-degrading species: bifidobacteria (7) and cluster IV ruminococci (23). Bifidobacteria showed a significant reduction with decreased carbohydrate intake, consistent with the previously reported impact of certain prebiotics on this group (28). In general, the findings discussed above in relation to the cluster XIVa bacterial group suggest that such diet-related population changes may become more apparent as probes become available that target smaller and functionally more coherent groups of bacteria. This might apply within the *Bacteroides* genus, for example, where species are known to differ with respect to carbohydrate-utilizing abilities (54).

The close correlation between the population densities of *Roseburia* and *E. rectale* species and fecal butyrate concentrations in response to altered carbohydrate supply supports a dominant role for these bacteria in butyrate production. Another group of butyrate producers, related to *F. prausnitzii*, was present in numbers approximately equal to those of *Roseburia* spp. and *E. rectale* at maintenance intake (13.5 versus 11.4% of total bacteria) but showed a weak correlation with fecal butyrate concentrations. This would indicate that this bacterial group makes a smaller contribution to fecal butyrate formation from carbohydrates in vivo, consistent with lower rates of butyrate production by *F. prausnitzii* compared with *Roseburia* strains in pure culture (17).

The observed changes in the populations of the *Roseburia* spp. and *E. rectale* group and in *Bifidobacterium* spp. may be a direct consequence of insufficient substrate to support growth, but this may not be the only cause. Studies in vitro using continuous flow fermentors inoculated with human fecal bacteria and supplied with a mixed polysaccharide energy source (mainly starch) produced substantial quantities of butyrate at pH 5.5 (60). Under these conditions the *Roseburia* spp. and *E. rectale* group, again monitored with the Rrec584 probe, represented approximately 20% of total bacteria. When the pH was increased to 6.5, however, this caused a fourfold drop in butyrate concentration, coupled with the virtual elimination of *Roseburia* spp. over a 9-day period (60), despite the continued supply of polysaccharides. In vivo, low pH (<6) is thought to accompany active carbohydrate fermentation in the proximal colon (10, 41), and this may allow *Roseburia* spp. to compete for carbohydrate substrates against other bacteria, such as *Bacteroides* spp., that are inhibited at low pH (60) and do not produce butyrate. Strategies to increase butyrate production within the large intestine therefore may depend, in part, on supplying sufficient fermentable carbohydrates from the diet (13) to maintain a mildly acidic pH in the lumen of the proximal colon.

The changes in fecal butyrate in the present study represent the largest reported in a human dietary trial and provide the strongest evidence to date that butyrate production is largely determined by the content of fermentable carbohydrate in the diet. Furthermore, this study has provided clear evidence that the proportions of certain groups of colonic bacteria, as monitored in fecal samples, respond to dietary carbohydrate intake. Whether or not the observed changes involving reduced SCFA formation, particularly that of butyrate, and altered microbial community profiles impact on colonic health cannot be assessed from this study. Nonetheless, accumulated evidence indicates that butyrate may promote apoptosis in colorectal cancer cells and help prevent colorectal cancer (4, 45, 47, 49, 59). In addition, an increased butyrate supply has been proposed to prevent colitis (35, 56). The optimal supply of butyrate required in the large intestine to maintain intestinal health is unclear, particularly as the effects on colonocyte cell biology in vivo are complex (12). The present study was of limited duration, and it is unknown whether the relatively short period of reduced butyrate and SCFA supply to the colonic mucosa would have long-term consequences for gut health. Such considerations may become important if low-carbohydrate diets are consumed for longer periods without ensuring that adequate forms of appropriate fermentable substrates comprise part of the diet.

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