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 their 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/m²) of 35.4 ± 0.9 (range, 30 to 42). Volunteers were selected...
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 periods at energy maintenance with two intervening main diet periods, at either low or moderate carbohydrate intake, each for 26 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).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Fat</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Nonstarch polysaccharides</th>
<th>Starch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maintenance</td>
<td>122.9</td>
<td>94.3</td>
<td>398.8</td>
<td>27.9</td>
<td>187.3</td>
</tr>
<tr>
<td>HPMC</td>
<td>74.3</td>
<td>127.2</td>
<td>163.6</td>
<td>11.7</td>
<td>95.3</td>
</tr>
<tr>
<td>HPLC</td>
<td>126.0</td>
<td>119.5</td>
<td>23.9</td>
<td>6.1</td>
<td>2.7</td>
</tr>
</tbody>
</table>

**P value**

- **<0.001**
- **0.001**
- **<0.001**
- **<0.001**

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

<table>
<thead>
<tr>
<th>Probe</th>
<th>Domain</th>
<th>Target</th>
<th>Reference for probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eub338</td>
<td>Bacteria</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Erec482</td>
<td>Clostridial clusters XIVa and XIVb</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Bac303</td>
<td>Bacteroides-Prevotella group</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Fpruau45</td>
<td>F. prausnitzii group (subgroup of cluster IV)</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Bif164</td>
<td>Bifidobacterium genus</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Rbro730/Rfla729</td>
<td>Ruminococcus bromii and Ruminococcus flavefaciens (subgroup of cluster IV)</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Rrec584</td>
<td>Roseburia and Eubacterium group (subgroup of cluster XIVa)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Lab158</td>
<td>Lactobacillus-Enterococcus group</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Prop853</td>
<td>Clostridial cluster IX</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Dsv698</td>
<td>Sulfate-reducing bacteria</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

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

**TABLE 3. Changes in fecal microbiota**

- **Ruminococcus bromii**
- **Ruminococcus flavefaciens**
- **Roseburia and Eubacterium group**
- **Lactobacillus-Enterococcus group**
- **Sulfate-reducing bacteria**

-Fig. 1: Fecal ammonia also declined with decreased carbohydrate intake (Table 3).

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Impact of Reduced Carbohydrate Intake in Obese Humans

Diet means were compared by Tukey’s test; different superscript capital letters within columns indicate P < 0.05.

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

<table>
<thead>
<tr>
<th>Diet</th>
<th>Formate</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Isobutyrate</th>
<th>Butyrate</th>
<th>Isovalerate</th>
<th>Valerate</th>
<th>Lactate</th>
<th>Succinate</th>
<th>Total SCFA</th>
<th>NH₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>0.56</td>
<td>65.09</td>
<td>20.28</td>
<td>2.27</td>
<td>17.67</td>
<td>2.13</td>
<td>3.14</td>
<td>1.23</td>
<td>1.27</td>
<td>113.6</td>
<td>51.67</td>
</tr>
<tr>
<td>HPMC</td>
<td>0.13</td>
<td>43.54</td>
<td>13.84</td>
<td>2.45</td>
<td>8.90</td>
<td>1.88</td>
<td>2.04</td>
<td>0.13</td>
<td>0.79</td>
<td>73.7</td>
<td>42.98</td>
</tr>
<tr>
<td>HPLC</td>
<td>0.27</td>
<td>35.50</td>
<td>10.81</td>
<td>1.91</td>
<td>4.36</td>
<td>1.34</td>
<td>1.38</td>
<td>0.00</td>
<td>0.63</td>
<td>56.2</td>
<td>33.03</td>
</tr>
</tbody>
</table>

SED: a n = 18 for M and HPLC diets; n = 16 for HPMC diet.

a 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.

b 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 clostridial cluster IV 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 10¹¹/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).

**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 intake (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-
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).

![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).](http://aem.asm.org/)

![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; XIaRa, Roseburia spp. and E. rectale, detected by Rrec584; XIa-R, clostridial cluster XIVa, detected by Rrec584; XIVa-R, clostridial cluster XIVa, detected by Rrec584 and Rbro730; IXIVp, F. prausnitzii detected by Fprau645; IX, clostridial cluster IX bacteria detected by Prop853; Bit, 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.](http://aem.asm.org/)
ference between studies may reflect either interindividual vari-
ation (the range was 6 to 21% for the current subjects) or, in light of the finding here, differences in the carbohydrate con-
tents 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 sig-
ificant reduction with decreased carbohydrate intake, consist-
tent 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 concentra-
tions in response to altered carbohydrate supply supports a dominant role for these bacteria in butyrate production. An-
other 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 bu-
utyrate 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 bu-
tyrate 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 bac-
teria 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, repre-
seated approximately 20% of total bacteria. When the pH was increased to 6.5, however, this caused a fourfold drop in bu-
utyrate 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 accom-
pany 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 Bac-
teroides 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 prox-
imal 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 mon-
tored 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 as-
essed from this study. Nonetheless, accumulated evidence in-
dicates that butyrate may promote apoptosis in colorectal can-
cer 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 re-
quired 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 dura-
tion, 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 con-
siderations may become important if low-carbohydrate diets are consumed for longer periods without ensuring that ade-
quate forms of appropriate fermentable substrates comprise part of the diet.

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