Acarbose Enhances Human Colonic Butyrate Production[1,2,3]

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ABSTRACT Earlier studies suggest that butyrate has colonic differentiating and nutritional effects and that acarbose increases butyrate production. To determine the effects of acarbose on colonic fermentation, subjects were given 50–200 mg acarbose or placebo (cornstarch), three times per day, with meals in a double-blind crossover study. Fecal concentrations of starch and starch-fermenting bacteria were measured and fecal fermentation products determined after incubation of fecal suspensions with and without added substrate for 6 and 24 h. Substrate additions were cornstarch, cornstarch plus acarbose and potato starch. Dietary starch consumption was similar during acarbose and placebo treatment periods, but fecal starch concentrations were found to be significantly greater with acarbose treatment. Ratios of starch-fermenting to total anaerobic bacteria were also significantly greater with acarbose treatment. Butyrate in feces, measured either as concentration or as percentage of total short-chain fatty acids, was significantly greater with acarbose treatment than with placebo treatment. Butyrate ranged from 22.3 to 27.5 mol/100 mol for the 50–200 mg, three times per day doses of acarbose compared with 18.3–19.3 mol/100 mol for the comparable placebo periods. The propionate in fecal total short-chain fatty acids was significantly less with acarbose treatment (10.7–12.1 mol/100 mol) than with placebo treatment (13.7–14.2 mol/100 mol). Butyrate production was significantly greater in fermentations in samples collected during acarbose treatment, whereas production of acetate and propionate was significantly less. Fermentation decreased when acarbose was added directly to cornstarch fermentations. Acarbose effectively augmented colonic butyrate production by several mechanisms; it reduced starch absorption, expanded concentrations of starch-fermenting and butyrate-producing bacteria and inhibited starch use by acetate- and propionate-producing bacteria. J. Nutr. 127: 717–723, 1997.

KEY WORDS: • *acarbose* • *starch* • *propionic acid* • *butyric acid* • *humans* • *colonic fermentation*

Acarbose is an oligosaccharide formed by strains of the genus *Actinoplanes* that functions as an α-glucosidase inhibitor (Merck Index 1983). It is effective for the treatment of diabetes because it slows the digestion of disaccharides and starch (Chiasson et al. 1994, Coniff et al. 1994 and 1995). Some carbohydrate, not digested in the small intestine, reaches the colon where it is exposed to microbial fermentation processes (Hiele et al. 1992, Wolever et al. 1995).

Starch-like carbohydrates can produce more microbial butyrate than other carbohydrates such as pectin or cabbage cellulose (Weaver et al. 1992). Butyrate production is of particular interest because it is partly responsible for maturation or differentiation of the rumen mucosa (Sakata and Tamate 1978, Sander et al. 1959, Van Soest 1982) and is a preferred colonocyte energy source (Roediger 1980). Colon cancer cell culture studies have shown that butyrate has a differentiating effect on malignant cells that includes the induction of proteins and peptide hormones, reversal of the morphology of transformed cells, formation of a normal cytoskeleton and arrest of cell growth in G_1 of the cell cycle (Kruh 1982). Specific examples of gene regulation by butyrate are the stimulation of fetal hemoglobin production by intravenous butyrate in humans (Perrine et al. 1993) and the reciprocal regulation of α-fetoprotein and albumin gene expression by butyrate in cultured hepatoma cells (Tsutsumi et al. 1994). Comparison of short-chain fatty acid (SCFA) concentrations in enema samples showed that the butyrate percentage of total SCFA was higher

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in subjects without colonic neoplasia compared with those with colonic neoplasia or a history of colonic neoplasia (Weaver et al. 1988). In another study (Kashtan et al. 1992), butyrate concentrations in feces were significantly higher in subjects without colonic polyps compared with those with colonic polyps. Fecal fermentation studies also support higher butyrate production in normal subjects compared with those with polyps (Clausen et al. 1991).

Scheppach et al. (1988) showed significant increases in the proportion of butyric acid in the SCFA of feces of subjects consuming high starch diets and acarbose. Acarbose probably augmented the fecal percentage of butyric acid by inhibiting starch digestion in the small intestine, thus allowing starch to reach the colonic microbial community for fermentation with preferential butyric acid production. Our study was conducted to determine the effects of acarbose on colonic microbial fermentation and to determine what dose of acarbose in subjects consuming a freely chosen diet would effectively raise colonic microbial butyrate production.

SUBJECTS AND METHODS

Study design. This study was conducted as a randomized double-blind placebo-controlled crossover trial. Volunteers were randomized to receive either acarbose (Bayer Corporation, West Haven, CT) or placebo (cornstarch). The initial dose of acarbose was 50 mg, three times per day, with meals. In the second week, the dose was 100 mg, three times per day, and in the third week, 200 mg, three times per day. After completion of the third week, the subjects received no study agent for 2 wk and then received placebo if they were initially randomized to acarbose, or acarbose if they were initially randomized to placebo, as in the initial 3 wk of the study. Subjects had physical examinations at the initiation and completion of each study period. Study visits also followed each weekly dose period at which time fecal samples and dietary records were collected and evaluated. Gastrointestinal entries were reviewed and scored retrospectively, without awareness of the study agent, for symptoms of rectal gas.

This study was reviewed and approved by the Institutional Review Board of The Mary Imogene Bassett Hospital on April 6, 1993.

Subject population. Subjects were volunteers and were excluded for any of the following reasons: antibiotic use within 1 mo; laxative use more than once a week; surgical removal of a portion of the intestinal tract other than the appendix; history consistent with inflammatory bowel disease; history of a bleeding disorder; pregnancy; age <21 y or >65 y; regular use of calcium supplements; use of more than 1.6 μmol of vitamin D per week; daily use of bile salts; and diabetes. Thirteen women and twelve men were enrolled in the study. Their ages ranged from 23 to 51 y with a mean age of 37 y. Two subjects were dropped from the study because of antibiotic use. A third subject was removed from the study due to *abnormal* liver tests. When the study code was broken, it was found that this subject had used only placebo. Data for one visit for one subject were lost because of a technical error. Thus the data from 22 subjects were available for most tests. Bacterial analyses were done in 17 subjects and starch analyses in 16 subjects.

Diet analysis. Subjects recorded what they ate during the 4 d before each study visit. These diet records were reviewed at each visit, and the edited dietary information was entered into the Nutritional Data System (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN). The average nutrient intake calculated from the Nutritional Data System for the 4 d records was used for subsequent analyses.

Fecal suspensions. Subjects voided fecal samples into polypropylene biohazard bags (Fisher Scientific, Pittsburgh, PA), closed the bags and placed them on ice in styrofoam coolers. Fecal samples were generally collected the evening before or morning of study visits. When subjects were unable to collect a sample in the 24 h before a study visit, samples were collected following the study visit but before an increase in the dose of the study agent. Because of the variation in physical consistency, varying amounts of neat feces were used to make suspension of ~100 g wet feces/L. Dry matter determinations (Weaver et al. 1986) were made on the final suspensions used for fermentation, and starch and bacterial analyses. Feces were transferred to stomacher bags and mixed by stomacher (Tekmar, Cincinnati, OH) with anaerobic dilution solution (Bryant and Burkey 1953) to make homogenous suspensions. When subjects were unable to void sufficient feces for a suspension of ~100 g wet feces/L, a more dilute suspension was used.

Fecal starch analysis. Ten milliliters of fecal suspensions was heated at 90–95°C for 45 min and digested with heat stable α-amylase (50 μL) (Sigma A-3306, Sigma Chemical, St Louis, MO) for 30 min. The samples were filtered and adjusted to 25 mL volume. Duplicate 5-mL aliquots were combined with 0.1 mol/L sodium acetate buffer, pH 4.0, (2 mL), digested with amyloglucosidase (66 U, Sigma A-3514) for 60 min, adjusted to volume and centrifuged at $3500 \times g$ for 4 min. Aliquots were analyzed for glucose with glucose oxidase (Sigma G-7016)/peroxidase (Sigma P-8125) reagents, and the resulting color read at $\lambda = 505$ nm. Controls (blanks with reagents and enzymes) were carried through the entire procedure (Karkalas 1985). Fecal carbohydrate concentrations are reported as micromoles of glucose per gram of dry matter with the assumption that most of the glucose came from fecal starch.

Bacterial analysis. Anaerobic conditions were maintained by use of the serum bottle-modification of the Hungate technique (Miller and Wolin 1974). The composition of the medium used for enumeration of total anaerobic bacteria in fecal samples as amount per liter was as follows: $NaHCO_3$, 7.5 g; K_2HPO_4, 0.3 g; KH_2PO_4, 0.3 g; $(NH_4)_2SO_4$, 0.3 g; NH_4Cl, 1 g: NaCl, 0.6 g; $MgSO_4 \cdot 7H_2O$, 0.12 g; $CaCl_2 \cdot 2H_2O$, 80 mg; $MgSO_4 \cdot 7H_2O$, 30 mg; $MnSO_4 \cdot H_2O$, 4.5 mg; $FeSO_4 \cdot 7H_2O$, 3.0 mg; $CoSO_4 \cdot 7H_2O$, 1.8 mg; $ZnSO_4 \cdot 7H_2O$, 1.8 mg; $CuSO_4 \cdot 5H_2O$, 100 μg; $AlK(SO_4)_2 \cdot 12H_2O$, 180 μg; $Na_2MoO_4 \cdot 2H_2O$, 100 μg; H_3BO_3, 100 μg; Na_2SeO_4, 1.9 mg; $NiCl_2 \cdot 6H_2O$, 92 μg; nitrilotriacetic acid, 15 mg; thiamine · HCl, 2.0 mg; D-pantothenic acid, 2.0 mg; nicotinamide, 2.0 mg; riboflavin, 2.0 mg; pyridoxine · HCl, 2.0 mg; biotin, 10.0 mg; cyanocobalamin, 20 μg; *p*-aminobenzoic acid, 100 μg; folic acid, 50 μg; L-cysteine · HCl · H_2O, 0.5 g; clarified rumen fluid, 100 mL; sodium acetate, 2.5 g; sodium formate, 2.5 g; yeast extract, 2 g; trypticase, 2 g; and agar; 20 g. Resazurin (1 mg/L) was added as an oxidation-reduction potential indicator. The same medium was used for the determination of starch-hydrolyzing bacteria except trypticase was omitted and cornstarch (10 g/L) was added.

The medium was dispensed in 3-mL amounts in 10-mL serum bottles and autoclaved under 100% CO_2 that had been passed through a heated column containing copper filings to remove trace amounts of oxygen. Before inoculation of roll tubes, the agar medium was melted and then cooled to 50°C; 0.15 mL of L-cysteine-sulfide 12.5 g/L solution and 0.1 mL of sterile solutions containing 0.9 g/L each of glucose, maltose and cellobiose were added.

Fecal suspensions prepared for enumeration of bacteria were shipped in refrigerated containers and analyzed 1 d after collection of fecal specimens. The suspensions were serially diluted (10-fold steps) in anaerobic dilution solution (Bryant and Burkey 1953), and 0.5 mL portions of appropriate dilutions were added to roll tubes for enumeration of total anaerobes and starch-hydrolyzing bacteria. After agar solidification, the tubes were incubated at 37°C. Duplicate portions of dilutions were used to enumerate bacterial colonies.

Starch hydrolysis was detected as clear zones surrounding colonies and monitored until the numbers of hydrolyzing colonies did not increase. Counts were usually completed within 1 wk after incubation. Total anaerobic bacteria were counted after 1 wk of incubation.

Total anaerobic bacteria and starch-degrading bacteria were estimated in 17 subjects. Bacterial counts were not done at every visit. To achieve sufficient samples ($n = 17$) for comparison, samples from the initial visit were grouped with samples from the placebo visits and averaged for each subject. Counts from available samples from all visits during acarbose treatment were averaged for each subject. These averaged values for each subject were compared by a paired *t* test.

Fermentations. Fermentations were conducted on fecal samples obtained at the completion of each dose period. Fermentations included the following: *1*) fermentation without added substrate (endogenous) for both 6 and 24 h, *2*) fermentation with 100 mg cornstarch (Sigma S-4126) for 6 and 24 h, *3*) fermentation with 100 mg

cornstarch plus 1 mg acarbose (Bayer Corporation) for 24 h, and 4) fermentation with 100 mg potato starch (Sigma S-2630) for both 6 and 24 h. Cornstarch and potato starch were used because they are common and have different characteristics. Because acarbose may have been excreted with the feces, fermentations with cornstarch were also conducted with added acarbose for comparison with cornstarch fermentations. Separate fermentations (single fermentation vials) for each time period (6 and 24 h) and substrate were conducted in sealed 20-mL serum bottles as previously described (Weaver et al. 1992). In brief, 5 mL of fecal suspension was added to 4.8 mL of phosphate and bicarbonate-carbon dioxide–buffered basal medium (pH 7.0) under a stream of 80% N_2:20% CO_2. The bottles were stoppered anaerobically and 0.2 mL of L-cysteine + $Na_2S \cdot 9H_2O$ (12.5 g/L of each) solution was added (Weaver et al. 1989). The bottles were incubated at 37°C for 6 or 24 h on a shaker. Reactions were terminated by boiling. Boiled samples without incubation were used to determine base-line concentrations of fermentation products.

Fecal and fermentation analyses. Gas liquid chromatography was used to determine concentrations of SCFA (principal SCFA: acetic, propionic and butyric; minor SCFA: isobutyric, isovaleric and valeric; total SCFA refers to principal plus minor fatty acids). Samples were acidified with sulfuric acid, centrifuged and filtered as previously described (Weaver et al. 1986). The gas liquid chromatography method, a modification of our previously described method (Weaver et al. 1989), used a Nukol fused-silica capillary column (15 m × 0.53 mm i.d. with a 0.5-μm film) (Supelco, Supelco Park, Bellefonte, PA). Injector and detector temperatures were 145 and 175°C, respectively. The initial oven temperature was 100°C and was increased by 10°C/min and then held at 130°C for 1 min. The carrier gas was helium with a flow of 8.7 mL/min. Sample injection volume was 1 μL. Chromatograms were integrated with a Perkin Elmer/Omega system (Norwalk, CT).

Headspace gas volume calculations were based on headspace pressure, fixed headspace volume, temperature and atmospheric pressure. Hydrogen was determined as previously described (Weaver et al. 1986) using a Gow-Mac Series 550P thermal conductivity gas chromatograph (Bound Brook, NJ) with a 1.8 m × 1 cm stainless steel column packed with 60/80 mesh silica gel (Alltech Associates, Deerfield, IL). The carrier gas was argon at a flow rate of 20 mL/min. Injector and column temperatures were 80°C, detector temperature was 30°C and bridge current was 90 mA. A 2-mL sample was used. Methane was determined as previously described (Weaver et al. 1986) using a Perkin-Elmer gas chromatograph with a flame ionization detector. A 1.8 m × 1 cm stainless steel column packed with molecular sieve 5A-60/80 mesh (Alltech Associates) was used. The carrier gas was nitrogen with a flow rate of 70 mL/min. Detector and injector temperatures were 175°C and oven temperature was 150°C. A 1-mL sample was used. Peaks for both hydrogen and methane were integrated with a Perkin Elmer/Omega system.

Fermentation product units. All fermentations with added starch contained the same dry matter amount of feces as that of their parallel fermentations without added starch. The products from 6 h fermentations are expressed on a micromoles per gram dry matter basis; because excess substrate was present at 6 h, the observed changes are not limited by substrate.

The data for 24-h fermentations with 100 mg of added substrate are expressed as micromoles of product produced in 24 h; the 24-h fermentations were sufficiently long for utilization of most of the added substrate.

Statistical methods. Values for sample size estimation were taken from the data of Scheppach et al. (1988) and from our data that showed constancy of SCFA percentages in samples from a given individual (Weaver et al. 1989). We estimated that the SD of the 9% rise in the butyric acid percentage of total SCFA in response to 200 mg of acarbose three times a day was ±8.4%. Based on this estimate, a sample size of 20 subjects would enable us to establish a 95% confidence interval around a mean rise in the butyric acid percentage of total SCFA of ±3.8%. Sample size was increased to 25 in anticipation of subjects leaving the experiment.

SAS (Statistical Analysis System, Version 6.08, SAS Institute, Cary, NC) procedures (General Linear Models procedure) were used for two- or three-way repeated measures ANOVA to examine treatment (acarbose or placebo), dose period (50, 100 or 200 mg, three times per day), and in vitro fermentation substrate (no added substrate or endogenous, 100 mg cornstarch, 100 mg cornstarch plus 1 mg acarbose, and potato starch (100 mg) effects. Type III sum of squares error terms were used for analyses except as noted. Post-hoc comparisons were done using Scheffe's test. Paired *t* tests were used to compare means of bacterial concentrations and ratios.

RESULTS

Dietary history comparison between acarbose and placebo treatment periods. Dietary variables calculated by the Nutritional Data System were alcohol, percentage of dietary protein, percentage of dietary fat, percentage of carbohydrate, calcium, selenium, cholesterol, total fiber, soluble fiber, insoluble fiber, pectin, starch, sucrose, galactose, glucose, fructose, lactose and vegetable protein. The only significant difference found between treatments was slightly greater lactose consumption during acarbose treatment periods (**Table 1**). Intakes of starch and sucrose are also shown in Table 1.

Clinical symptoms. The only clinical symptoms noted by subjects taking acarbose occurred in the gastrointestinal system. The most frequent difference in symptoms reported was greater rectal gas while using acarbose. Rectal gas scores indicated significantly greater passage of gas during acarbose treatment. The number of bowel movements per week was significantly higher when the dose of acarbose was 200 mg compared with placebo (Table 1).

Fecal starch. Fecal starch concentrations were measured at the completion of the 200-mg dose period in 16 subjects. The concentration after the 200-mg acarbose period was significantly greater than after the placebo period (Table 1). Small amounts of the starch could have been from glucose and oligosaccharides derived from starch that escaped bacterial consumption.

Starch-fermenting bacteria. Total anaerobic bacterial counts for subjects not taking acarbose (means of available initial visit and placebo visit values) of 11.31 $\log_{10}$/g dry matter were similar to counts for those taking acarbose of 11.33 $\log_{10}$/g dry matter (means of available values for 100- and 200-mg acarbose doses). The counts of starch-fermenting bacteria during placebo and initial visits (10.78 $\log_{10}$/g dry matter) were not significantly different from those during acarbose treatment (10.97 $\log_{10}$/g dry matter). However, the starch-fermenting bacteria percentage of total viable anaerobes during acarbose treatment was significantly greater than during placebo treatment or initial visits (Table 1). This is consistent with adaptation of the microbial population to a higher colonic starch load.

Fecal SCFA concentrations. Concentrations of fecal SCFA are shown in **Figure 1**; also included are trace amounts of gas, from feces and brief fermentation before bacteria were killed by heat, released into the headspace of the suspension vial after boiling. Butyrate concentrations, but not those of the other SCFA, were significantly higher during acarbose treatment than during placebo treatment (Fig. 1). Butyrate levels as a percentage of total SCFA (Table 1) were significantly greater for acarbose treatment than for placebo treatment. Fecal propionate percentages were significantly less during acarbose treatment than during placebo treatment (Table 1).

Products of in vitro fermentation. Butyrate and hydrogen production, (μmol/g dry matter) were significantly greater after 6 h of fermentation (Fig. 1) for samples obtained during the acarbose treatment compared with those obtained during the placebo treatment. Significant treatment effects were not seen for acetate or propionate concentrations. Butyrate as a percent-

TABLE 1

Dietary carbohydrates, bowel movement frequency, fecal starch, fecal starch degrading bacteria percentage of total anaerobic bacteria, and fecal short-chain fatty acid percentages in human subjects taking acarbose or placebo (cornstarch)[1] three times per day

Variable	Placebo			Acarbose		
Dose	50 mg	100 mg	200 mg	50 mg	100 mg	200 mg
Oral intake, *g/d*						
Starch	130.2 ± 10.4	129.8 ± 10.4	113.1 ± 7.9	128.6 ± 10.9	119.5 ± 8.6	122.1 ± 9.8
Sucrose	53.0 ± 10.4	60.1 ± 5.6	54.1 ± 5.4	62.2 ± 5.3	52.8 ± 3.9	56.4 ± 5.7
Lactose[2]	17.4 ± 2.2	15.8 ± 2.2	19.0 ± 3.2	20.2 ± 2.7	18.6 ± 2.7	20.7 ± 3.1
Output						
Bowel movements, *n/wk*[3]	*9.3 ± 1.4*	*8.1 ± 1.3*	*8.3 ± 1.3*	*10.5 ± 1.7*	*11.3 ± 1.9*	*16.3 ± 2.5*
Fecal starch, *μmol/g dry matter*[4]			68.5 ± 12.5			241.2 ± 62.9
Starch fermenting bacteria,[5] %			31.1 ± 3.4			49.0 ± 6.3
			mol/100 mol of total SCFA			
Fecal SCFA						
Acetate	59.0 ± 1.4	59.0 ± 1.2	58.5 ± 1.2	59.3 ± 1.7	57.2 ± 2.6	56.6 ± 2.5
Propionate[6]	14.2 ± 0.8	14.7 ± 0.8	13.7 ± 0.7	12.1 ± 0.9	11.2 ± 0.8	10.7 ± 0.8
Butyrate[6]	18.3 ± 1.0	17.9 ± 1.2	19.3 ± 1.3	22.3 ± 2.0	25.4 ± 3.0	27.5 ± 3.0

[1] Values are means ± SEM; oral intake, bowel movements and fecal SCFA as mol/100 mol of total SCFA, $n = 22$ for 50- and 100-mg dose periods and $n = 21$ for the 200-mg dose period; fecal starch, $n = 16$; % starch fermenting bacteria, $n = 17$.

[2] Acarbose treatment values were significantly different than placebo treatment values by ANOVA, $P = 0.03$.

[3] Acarbose 200-mg treatment values were significantly different than 200-mg placebo treatment values by ANOVA (type II sum of squares) and post hoc Scheffé's test, $P = 0.012$.

[4] Acarbose treatment values were significantly different than placebo treatment values by ANOVA, $P = 0.024$.

[5] Starch-utilizing bacteria as percentage of total anaerobic bacteria. (Placebo mean is calculated from placebo visits and visits prior to treatment. Acarbose treatment means are from available values during acarbose treatment periods.) Acarbose treatment values were significantly different than placebo treatment values by a paired *t* test, $P = 0.025$.

[6] Acarbose treatment values were significantly different than placebo treatment values by ANOVA, $P = 0.0001$.

age of total SCFA after 6 h of endogenous fermentation was significantly greater and propionate significantly less during acarbose treatment than during placebo treatment. Acetate percentages for endogenous fermentations were not significantly different between treatments.

Six hour fermentation with cornstarch or potato starch. Fermentation products were measured in fecal suspensions with added cornstarch and added potato starch after 6 h. These results, again expressed as micromoles per gram dry matter, are shown in **Figure 2.** Total SCFA production did not differ between acarbose and placebo treatment. However, the production of butyrate and propionate did differ significantly between the two treatments. Propionate production was greater with placebo treatment for both the cornstarch and the potato starch fermentations. Butyrate production was greater during acarbose treatment for the cornstarch fermentation; the ANOVA Substrate × Treatment term for butyrate was significant, and post-hoc testing showed that during acarbose treatment, butyrate concentrations were significantly greater for cornstarch fermentations compared with placebo treatment. Butyrate formed from potato starch did not differ significantly between acarbose and placebo treatments. When 6 h endogenous values (Fig. 1) were subtracted from 6 h starch fermentation values (Fig. 2), butyrate production from cornstarch remained significantly greater with acarbose treatment ($P = 0.021$). This suggests that the capacity for butyrate production was greater with acarbose treatment and that the greater butyrate production during acarbose treatment was not due to more endogenous starch in the acarbose treatment samples compared with the placebo treatment samples. After endogenous propionate values (Fig. 1) were subtracted from cornstarch fermentation values, the significant differences in propionate production between acarbose and placebo treatment samples remained. That is, significantly less propionate was produced from cornstarch fermentation during acarbose treatment. This suggests that an inhibitory effect of residual acarbose in the fecal samples rather than a difference in endogenous substrate composition caused the differences in propionate production between samples taken during acarbose and placebo treatment. Potato starch was fermented more slowly than cornstarch with significantly less production of each product in 6 h (Fig. 2). The lesser production of SCFA from potato starch may have masked potential differences in butyrate production between treatments. The production of SCFA from potato starch at 6 h was 1.4 times the endogenous production, whereas the production from cornstarch after 6 h was 2.5 times the endogenous production.

Butyrate percentage of total SCFA at 6 h was significantly greater during acarbose treatment than during placebo treatment for both the cornstarch and potato starch fermentations. Because starch substrate was not limiting for the 6-h fermentations, the greater percentage of butyrate suggests an increased propensity for butyrate formation with acarbose treatment.

Effect of acarbose added to in vitro cornstarch fermentation. The 24-h products from fermentations of cornstarch and cornstarch with added acarbose are shown in **Figure 3.** Separate three-way ANOVA (treatment, substrate, dose) for the fermentation products (total μmol) showed significantly less acetate production and significantly more butyrate production during in vivo acarbose treatment. When acarbose was added to the in vitro fermentations, significantly less acetate, propionate and butyrate were produced. Less hydrogen was also pro-

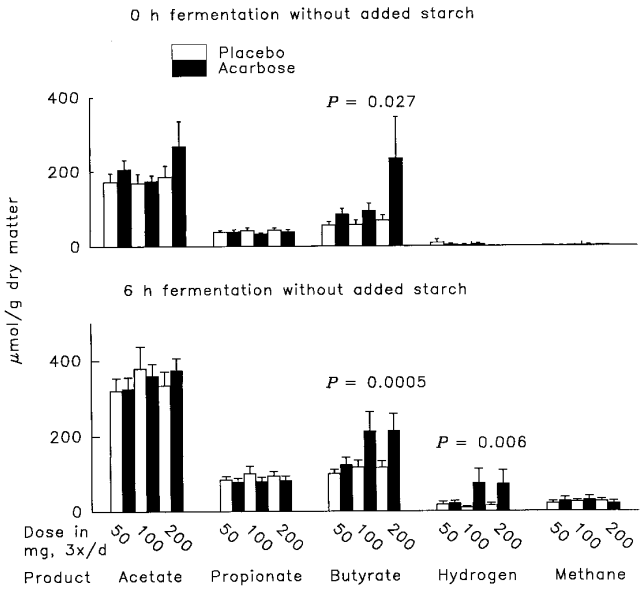

FIGURE 1 Short-chain fatty acids, hydrogen and methane from base line (0 h) and 6 h in vitro fermentation of human fecal suspensions without added substrate. Subjects were treated with acarbose and placebo. Values are means ± SEM, $n = 22$ except for acarbose 200-mg dose, $n = 21$. Paired bars (placebo treatment and acarbose treatment) show products for each of the three dose periods of 50, 100 and 200 mg, three times per day. The *upper panel* shows base-line values without incubation; the *lower panel* shows values after 6 h of incubation. *Y*-axis intervals are the same for each panel of this figure and Figure 2 for comparison. Two-way repeated measures ANOVA results for each time (panel) and product for treatment effect (placebo, acarbose) are shown on the graphs. Dose effect was significant for butyric acid for the 6-h fermentation ($P = 0.05$).

duced when acarbose was added to the in vitro fermentations. Propionate production was significantly higher during placebo treatment than acarbose treatment for cornstarch fermentations without acarbose added to the in vitro fermentations, but when acarbose was added to the in vitro cornstarch fermentations, propionate production did not differ between samples taken during acarbose and placebo treatments. Taken together, these results are consistent with in vivo and in vitro inhibition of starch fermentation by acarbose.

Comparison of fermentation product differences between substrates. Fermentations of cornstarch yielded significantly more SCFA, hydrogen and methane at 6 h than did those of potato starch (Fig. 2). Fermentations of cornstarch for 24 h with or without added acarbose (Fig. 3) and of endogenous substrate or potato starch (data not shown) showed significant differences in the relative production of the SCFA. Cornstarch showed the highest percentage of total SCFA (least square mean) of butyrate production (30.3%, range for other substrates 21–29.9%), potato starch the highest percentage (least square mean) of acetate production (60.1%, range for other substrates 55.8–57.2%), and endogenous substrate the highest percentage of propionate production (15.5%, range for other substrates 8.7–10.0%).

DISCUSSION

Acarbose, administered to human subjects at a maximum dose of 200 mg, three times per day, was associated with several changes in the composition of subjects' feces. The amount of starch that escaped digestion in the upper gut rose from 0.33 to 1.17 g/d (estimates based on an output of 30 g dry matter/d and values in Table 1) with an intake of 110–130 g/d. The proportion of starch-using bacteria rose from 31 to 49% of total fecal anaerobes. Finally, the concentration of butyrate rose from 56 to 235 μmol/g dry matter of feces.

When feces were incubated under anaerobic conditions at 37°C for 6 h without added substrate, butyrate production increased over basal fecal values. Butyrate concentrations were greater at 6 h from subject samples collected during acarbose treatment at 100 or 200 mg, three times per day, than during placebo treatment. The pattern of short-chain fatty acid production during 6 h of in vitro fermentations was very similar to the pattern found in the feces; thus, in vitro fermentation appeared to parallel the in vivo microbial fermentation of malabsorbed dietary substrate. Addition of cornstarch to the incubations resulted in a further increase in butyrate production, which was also greater in samples from acarbose-treated subjects (50–200 mg, three times per day) than in samples taken during placebo treatment.

Acarbose added to starch-supplemented fermentations

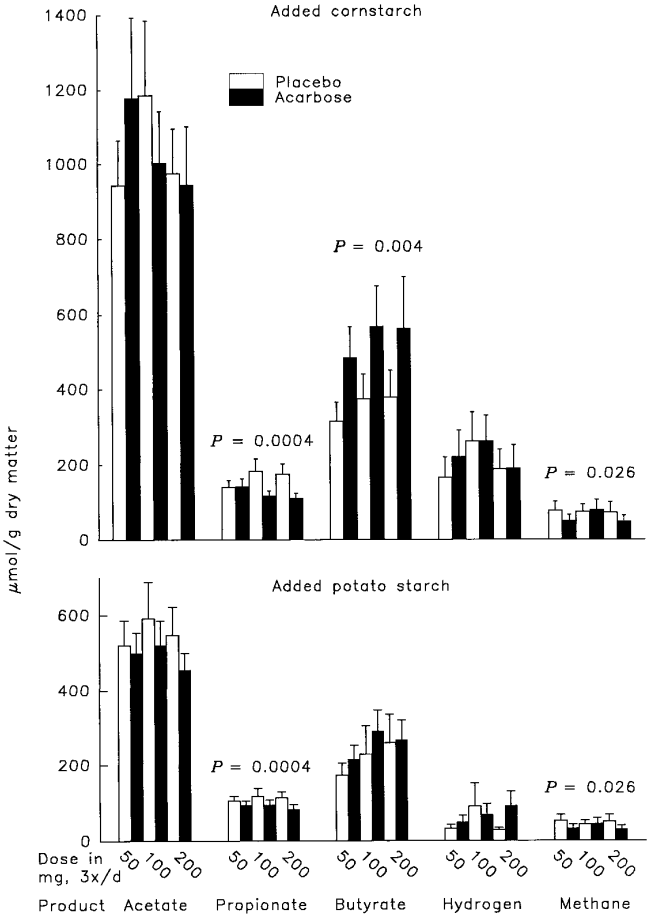

FIGURE 2 Short-chain fatty acids, hydrogen and methane from 6 h in vitro fermentation of human fecal suspensions with 100 mg of cornstarch (*upper panel*) and 100 mg of potato starch (*lower panel*). Subjects were treated with acarbose and placebo. Values are means ± SEM, $n = 22$ except for acarbose 200-mg dose, $n = 21$. Paired bars (placebo treatment and acarbose treatment) show products for each of the three dose periods of 50, 100 and 200 mg, three times per day. *Y*-axis intervals are the same for each panel of this figure and Figure 1 for comparison. Three-way repeated measures ANOVA results for each product for treatment effect (placebo, acarbose) are shown on the graphs. Substrate effect differences were significant for each product ($P = 0.0001$). The Treatment × substrate interaction was significant for butyrate ($P = 0.047$).

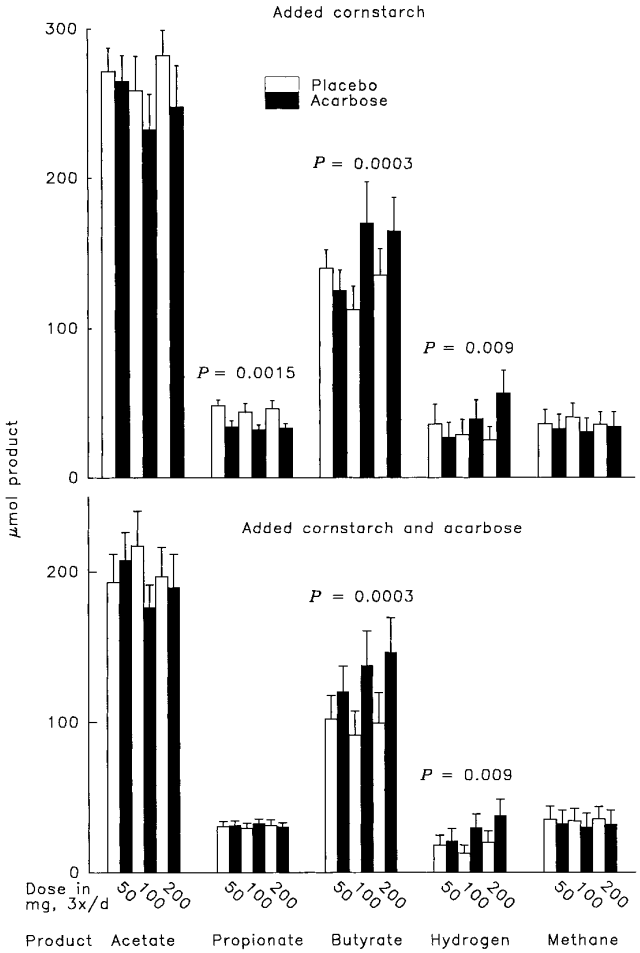

FIGURE 3 Short-chain fatty acids, hydrogen and methane from 24 h in vitro fermentation of human fecal suspensions with 100 mg of cornstarch plus 1 mg of acarbose (*lower panel*) and 100 mg of cornstarch (*upper panel*). Subjects were treated with acarbose and placebo. Values are means ± SEM, $n = 22$ except for acarbose 200-mg dose, $n = 21$. Paired bars (placebo treatment and acarbose treatment) show products for each of the three dose periods of 50, 100 and 200 mg, three times per day. *Y*-axis intervals are the same for each panel. Results of three-way repeated measures ANOVA for each product for treatment effect (acarbose and placebo in vivo) are shown on the graphs. Substrate effects (cornstarch, cornstarch plus acarbose) were significant for acetate ($P = 0.0001$), propionate ($P = 0.0001$), butyrate ($P = 0.0019$) and hydrogen ($P = 0.0044$) but not methane.

caused a reduction in short-chain fatty acid production over 24 h. The reduction in acetate from acarbose added to the in vitro fermentations was similar in samples taken during placebo and acarbose treatment. In contrast, acarbose addition to in vitro fermentations caused a greater propionate reduction in samples taken during placebo treatment than in samples taken during acarbose treatment. The absence of a reduction in propionate from acarbose added to in vitro fermentations of samples taken during acarbose treatment suggests that propionate production was already suppressed in these samples by in vivo effects of acarbose or residual acarbose in the fecal sample. Lower fecal propionate percentages during acarbose treatment also suggest in vivo inhibition of propionate production. Although acarbose added to the in vitro fermentations reduced butyrate production from samples taken during acarbose and placebo treatment, samples taken during acarbose treatment continued to produce more butyrate than samples taken during placebo treatment. This could be explained by higher concentrations of butyrate-producing bacteria during acarbose treatment and higher concentrations of starch in the samples taken during acarbose treatment.

The major mechanism causing greater fecal starch during acarbose treatment would be expected to be the well-known inhibitory action of this drug toward human amylase, but inhibition of bacterial amylases should also be postulated to allow passage of starch through the colon. Another factor could be the increase in frequency of bowel movements that occurred with acarbose treatment. This would restrict the time for digestion and microbial activity.

This study confirms that acarbose enhances colonic butyrate production. Each dose of acarbose (50, 100 and 200 mg, three times per day) effectively increased butyrate production. Because of the increased frequency of bowel movements seen with the 200-mg dose, the optimal dose to increase fecal butyrate is 100 mg.

Our studies are consistent with the following changes occurring when subjects switched from placebo to acarbose: enhanced butyrate production from a higher ratio of starch-hydrolyzing to total anaerobic bacteria in feces because of reduced digestion of starch and inhibition of some aspects of bacterial fermentation shown by reduced propionate production. Our studies also showed that potato starch was more resistant to fermentation than cornstarch, suggesting that other starches resistant to digestion may also be resistant to fermentation.

Because of the propensity of colonic starch fermentation to yield butyrate and because of the interest in the health benefits of butyrate, identification of the predominant species of starch-fermenting bacteria that form butyrate merits investigation. It appears that substantial concentrations of these bacteria are present in the colon and that the amount of butyrate they form is limited by the availability of starch as a fermentation substrate.

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