# Effects of Acarbose on Fecal Nutrients, Colonic pH, and Short-Chain Fatty Acids and Rectal Proliferative Indices

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**Acarbose, an α-glycosidase inhibitor, treats diabetes mellitus by delaying the digestion and intestinal absorption of dietary carbohydrates. In effective doses, acarbose induces some passage of carbohydrates into the colon. The effect of such chronic carbohydrate transfer on colonic structure and function is unknown. We studied the effects of 1 year of acarbose administration in diabetes mellitus on fecal energy, protein, and fat, including short-chain fatty acids (SCFA) output, fecal pH, and several metabolizing bacterial species. Changes in colonic histology and epithelial cell proliferation were investigated in rectal biopsies. Fecal macronutrient output was unaffected by acarbose, but pH decreased and total SCFA, butyrate, and acetate output were markedly greater. Breath hydrogen output increased after acarbose, but digoxin-metabolizing bacteria and diacylglycerol (DAG) production were unaltered. Compared with the control, acarbose did not induce hyperplasia or change rectal proliferation. However, total fecal SCFA and butyrate output correlated inversely with proliferation in the rectal upper crypt—a biomarker of risk for colonic neoplasia. In conclusion, long-term acarbose administration does not adversely affect colonic function or fecal nutrient output. If increased fecal SCFA and butyrate reduces upper-crypt proliferation, then acarbose may reduce the risk of colonic neoplasia.**



RECENTLY, a novel group of therapeutic agents have been added to the treatment of diabetes mellitus. These are enzyme inhibitors that impede the hydrolysis of polysaccharides and disaccharides to absorbable monosaccharides in the intestinal lumen. They delay the absorption of carbohydrates in the proximal small intestine and thereby reduce postprandial blood glucose profiles. Acarbose, a mostly nonabsorbable inhibitor of intestinal sucrase-isomaltase and intraluminal amylase activity at physiologic substrate concentrations,[1] has been used successfully in Europe[2] and Canada[3] to treat diabetes mellitus. The drug recently has been approved for use in the United States by the Food and Drug Administration. The tolerable dose of α-glycosidase inhibitors is determined by abdominal symptoms[4] resulting from the passage of unabsorbed carbohydrate into the colon. Unabsorbed carbohydrate in the colon is metabolized to osmotically more active carbohydrate metabolites such as monosaccharides, which then can be further broken down to short-chain fatty acids (SCFA) and gases such as hydrogen and methane.[5] These metabolites may themselves directly affect colonic structure and function.

Since the potential long-term consequences of such carbohydrate malabsorption and metabolism are not known, the present studies were designed to determine the effects of acarbose on fecal output of energy and several fecal components including pH, fat, nitrogen, and SCFA. In addition, we analyzed the potential effects of acarbose treatment on colonic bacteria by determining changes in breath hydrogen output, digitalis-metabolizing bacteria concentration, and diacylglycerol (DAG) production. Furthermore, because of the putative antineoplastic effects of SCFA, the consequences of long-term acarbose administration for several proliferative markers were also determined in biopsies obtained at the start and end of 1 year of treatment.

## SUBJECTS AND METHODS

The patients (volunteers) that participated in this study were a subgroup of individuals being studied nationally to evaluate the long-term safety and tolerance of acarbose in patients with diabetes mellitus. This study was conducted at two centers, St. Luke's-Roosevelt Hospital Center in New York City and Minneapolis Veterans Administration Hospital in Minneapolis, MN. These diabetic patients were receiving diet therapy alone or oral antidiabetic medications and/or insulin therapy. At these two centers, consecutive subjects who did not have treatment contraindications and who agreed to participate were entered. All subjects signed a separate informed-consent form. Twenty-seven subjects were entered from the St. Luke's site and 18 from Minneapolis. The patients did not have a personal or family history of colon neoplasia, and were randomly assigned to treatments such that 15 of these 45 subjects received placebo pills formulated to be identical to the study medication and 30 received acarbose at a dose varying from 50 to 300 mg three times daily. At the onset of the study, acarbose-treated subjects received 50 mg of the drug three times daily taken with the first bite of each meal. After 2 weeks, the same dosing regimen of acarbose was increased by titration to 100 mg three times daily, to 200 mg three times daily 2 weeks later, and, in some subjects, to 300 mg three times daily 2 weeks after that (Fig 1). Titration was based on tolerance. For the remainder of the study, each subject received their maximum tolerated dose. Placebo-treated subjects received an identical dummy pill in this double-blind study.

The study was approved by the Institutional Review Board of St. Luke's-Roosevelt Institute of Health Sciences on December 19, 1990, and by the Human Studies Committee of the Minneapolis Veterans Administration Medical Center on January 28, 1991.

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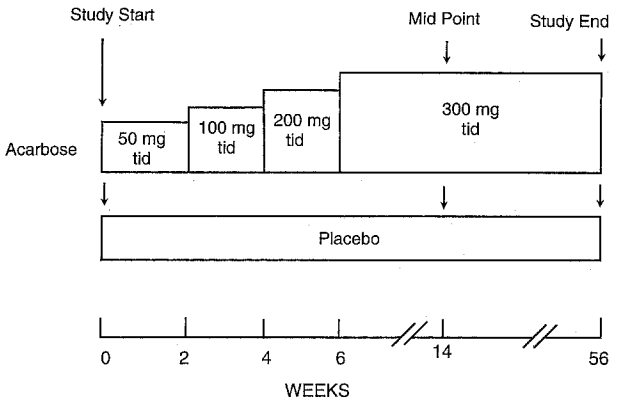


**Fig 1. Diagram of overall design of the study.**

## *Overall Methodology*

The analyses were performed on all subjects at entry to the study and at study completion after 56 weeks of treatment (Fig 1). In addition, several fecal components were evaluated at an intermediate time point (midpoint) approximately 14 weeks after the start of the study, at least 6 to 8 weeks after the acarbose-treated subjects reached their maximum dose of medication. At study start and study end, a 3-day stool sample was collected for quantitative analysis of fecal-component concentrations and outputs, and a rectal biopsy and breath hydrogen testing were performed. At midpoint, a single stool sample was collected for analysis of concentrations of fecal components, and breath hydrogen level was also measured to determine hydrogen output.

## *Detailed Methodology*

*Quantitative fecal output.* A 3-day stool collection was performed in the home as previously described[6] at study start and study end. The patients were provided a disposable fecal collection apparatus that is attachable to toilet seats. Feces were collected directly into plastic bags attached to the toilet collection apparatus. After each bowel movement, the stool was double-bagged and immediately placed into a container with dry ice. The subjects were sent dry ice containers on the afternoon before beginning the 3-day stool collection, with sufficient ice to last for more than 3 days. Each stool sample was separately placed into the container, which was returned to the laboratory as soon as the 3-day collection was completed. The stools were then kept at −80°C until analysis. The single stool sample at the study midpoint was brought to the laboratory within 1 hour of collection.

*Breath hydrogen determination.* End-expiratory breath hydrogen concentrations were determined twice during fasting and then hourly for 8 hours, as previously described from this laboratory,[6,7] after ingestion of 2 tablets of acarbose (100 mg) and a standard breakfast. The standard breakfast consisted of 4 oz of orange juice, 2 slices of whole wheat bread, 2 teaspoons of margarine, 1 oz of oatmeal, and 1 slice of American cheese, and contained 371 kcal with 55.4% as carbohydrate, 10.3% as protein, and 34.3% as fat. On the evening before breath hydrogen analysis, subjects were instructed to take small amounts of carbohydrates, in the form of complex carbohydrate only, in the evening meal. To avoid hypoglycemia, the patient's normal dose of antidiabetic medication was reduced by one third and taken 15 minutes before the standard breakfast. In addition, subjects were permitted to eat a small standard lunch consisting of 1 cup of creamed cottage cheese, 4 oz of water, and packed peaches (250 kcal energy: 21% as carbohydrate, 44% as protein, and 35% as fat) approximately 5 hours after initiation of the breath hydrogen test. Breath hydrogen level was measured by gas chromatography, and both peak concentration and integrated output over the 8-hour period were determined.[6]

*Rectal biopsy procedures.* Flexible sigmoidoscopy was performed between 8 and 10 AM, and the fasting subjects were prepared for the procedure with a tap water enema (60 mL). A total of four to six biopsies were obtained in four quadrants of the rectum 10 to 15 cm from the anal verge. Biopsies were immediately placed into gassed Eagle's minimal essential transport medium, and within 10 minutes were cut into 2 × 3-mm sections, oriented, and incubated for 90 minutes in Eagle's minimal essential culture medium with 10% fetal calf serum containing 5 μCi $^3$H-TdR/mL as previously described.[8]

## *Details of Chemical Methods*

*Quantitative output of fecal components.* The fecal collection was weighed, and aliquots of the frozen stool were taken to determine water content, pH, digoxin-metabolizing bacteria, DAG, and SCFA composition. Initial analyses showed that there were no significant differences in these determinations in samples taken from different parts of the stool. However, in general, two or three portions of the stool were taken and analyzed together. The remainder of the stool was defrosted, diluted with an equal quantity of water, and homogenized by standard methods in a paint shaker. Aliquots of the homogenized stool were dried again to constant weight to determine water content. Total fat was determined by gravimetric analysis,[9] total nitrogen by a micro-Kjeldahl technique (total protein content estimated by multiplying by a factor of 6.25),[10] and total energy by bomb calorimetry.[11]

Total daily output of energy, fat, and protein was calculated by multiplying the calorie, protein, or fat content of the weighed aliquot by the mean fecal excretion per day. The output of other components—presumably principally carbohydrate from fiber and cell wall components—was determined by subtracting the calorie content of fecal fat and protein from total fecal calorie output.

pH was determined in duplicate or triplicate using a micro-type probe; digoxin-metabolizing bacteria as previously described[12,13]; DAG by lipid extraction, thin-layer chromatography, and densitometry as previously described from this laboratory[14]; and total and individual SCFA by gas chromatography.[15] Briefly, samples were placed in liquid nitrogen and ground to a fine powder with a chilled mortar and pestle. Approximately 0.3 g powdered sample was weighed accurately into a 1.5-mL microcentrifuge tube, to which 500 μL 3-mmol/L 2-ethyl butyric acid in 70% ethanol was added as internal standard. Each tube was vortexed and stored at 4°C overnight. Afterward, the tubes were shaken for 20 minutes. Samples were then centrifuged for 20 minutes at 11,500 × *g* at 4°C. After centrifugation, the resulting supernatant was combined with a second internal standard (heptanoic acid). Immediately before injection, 20 μL 10% $H_3PO_4$ was added to each vial, and 1 μL was injected onto the gas chromatograph. Fatty acids were separated using a Hewlett Packard 589 Series II Gas Chromatograph (Corvallis, OR) with a flame ionization detector equipped with a 30-m, 0.53-mm ID, HPFFAP-TPA capillary column and a 1-m, 0.53-mm ID deactivated glass capillary precolumn. Chromatography conditions were as previously described.[15] SCFA standards containing 2-ethyl butyric acid, heptanoic acid, and $H_3PO_4$ were chromatographed at the beginning and end of daily sample analyses. Blanks containing only internal-standard solutions in 70% ethanol were also chromatographed daily. Response factors and retention times for individual fatty acids were determined from results of the daily standard analyses. Concentrations of SCFA were calculated by comparing peaks with known standards and correcting for recovery.

*Rectal Biopsy Proliferative Kinetics*

Specimens were incubated in a moistened atmosphere of 95% $O_2$ and 5% $CO_2$ at 2× atmospheric pressure at 37°C in the $^3$H-thymidine-containing medium in a rocking platform chamber. They were then washed, oriented, dehydrated, and fixed in 10% Formalin (three to four specimens) and/or 95% ethanol (two specimens). The specimens were then handled by routine histopathological methods, cut at 4-μm thickness, and processed for autoradiography.[8] Twelve to 28 crypts were counted per biopsy specimen. The positions of $^3$H-labeled cells within colonic crypt columns were standardized in each subject, and the height of labeled epithelial cells was measured and expressed in a uniform manner.[16] For measuring the distribution of labeled cells, each labeled cell was assigned a crypt coordinate value between 0 and 1 and equal to the fraction of the location of the cell above the base of its crypt column.[16] Labeling of the upper crypt, which is one index of neoplastic risk, was evaluated by determination of labeling of nuclei within compartment 4 (crypt coordinate value, 0.61 to 0.80) and Φ h.[16] Φ h is a measure of the percent of proliferating cells in the upper 40% of the crypt.

*Statistical Methods*

All tests were two-tailed and performed at the 5% significance level. Analyses were performed with SAS (Cary, NC) version 6.04 PROC *t* test, with experimental group (acarbose or control) as the CLASS variable. The *t* tests were performed on all available measures at each visit for all three of the raw concentration, total output, and chromium-adjusted values. To test the difference between experimental groups or the change between two time points, a difference score was calculated with the earlier time point value subtracted from the later time point value: thus, the study end propionic acid concentration minus the study start propionic acid concentration became the study end to beginning propionic acid concentration difference score. Again, experimental group formed the SAS CLASS variable for the comparison of difference score means for the two groups. All *P* values are reported after undergoing evaluation for equality of variances according to the SAS method of calculating a two-tailed folded F test. For variances with this F test yielding a *P* value for the equality of variances greater than 5%, the *P* value associated with the *t* test assuming equal variance is reported. For variances with this F test producing *P* values less than 5%, the *P* value associated with the test comparing means uses a test adjusted for unequal variances by reducing the degrees of freedom for the critical *t* value according to methods attributed by the SAS manuals to Satterthwaite.[17] Within-group changes were evaluated with *t* tests and repeated-measures ANOVA.

## RESULTS

*Patients*

All 15 placebo-treated subjects and 24 of 30 subjects receiving acarbose completed the 12-month study. Three acarbose-treated subjects withdrew from the study between the start and the midpoint collection, and the other three patients withdrew between the midpoint and end-point collection. The reasons for withdrawing from the study for five subjects were medication side effects, principally excessive flatus and/or abdominal cramps, and, for one, a coincidental illness. Symptom complaints were greater with increasing doses of acarbose.

*Fecal Weight, Water Content, and pH*

In placebo subjects, neither fecal wet weight (Fig 2) nor dry weight differed significantly in the 1-year study period. In acarbose-treated subjects, wet weight increased by 43% (155 *v* 221 g, $P < .01$; Fig 2) and dry weight by 37% (56.7 *v* 41.3 g, $P < .01$; data not shown). In acarbose-treated subjects, there was a significant increase in mean fecal water output, from 114 ± 10.5 to 166 ± 20.6 mL/d ($P < .05$). At study start, fecal pH was identical in the two groups (pH 6.9; Fig 3). At the midpoint, the mean fecal pH in acarbose-treated subjects was less than in placebo subjects, but this did not reach significance ($P < .08$). At the end point, fecal pH in acarbose subjects was 6.3 ± 0.1 and in controls 7.0 ± 0.1 ($P < .001$). Analysis of differences in pH also showed a significant difference only between the midpoint and final end-point collection.

*Fecal Excretion of Macronutrients*

Macronutrient excretion was calculated as calorie, fat, and nitrogen content and output and as "other" determined by subtracting the calculated fecal energy from fat and nitrogen output from total fecal calorie output. There were no significant differences in the concentration of fecal components per gram fecal dry weight.

Daily fecal output of energy, nitrogen, fat, or "other components" did not differ at study start between placebo- and acarbose-treated subjects (Fig 4). At study end, output of total energy and nitrogen was slightly higher in acarbose-treated subjects than in those receiving placebo, but these differences did not reach significance (Fig 4). Furthermore, calculated differences in individual subjects across the study also did not differ significantly between placebo- and acarbose-treated subjects.

*Fecal SCFA Output*

There were no significant differences in fecal total SCFA concentrations between placebo- and acarbose-treated subjects at any time (Table 1). There also were no differences in total daily SCFA output between placebo- and acarbose-treated subjects at study start, but total outputs in acarbose-treated subjects at study end increased by 212% and were significantly different ($P < .01$; Fig 5).

Analysis of individual SCFA showed no differences in

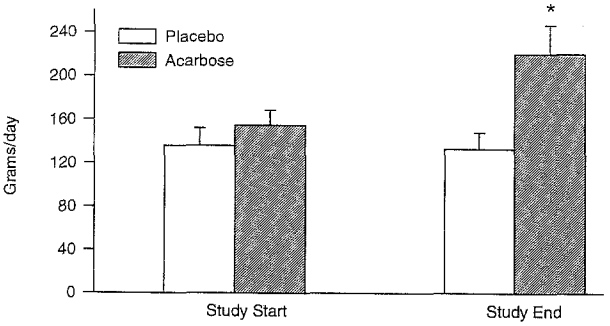


**Fig 2. Fecal wet weight in placebo- and acarbose-treated subjects. Data are presented as mean fecal weight with bars designating SE in all subjects at the time points shown. *$P < .05$.**

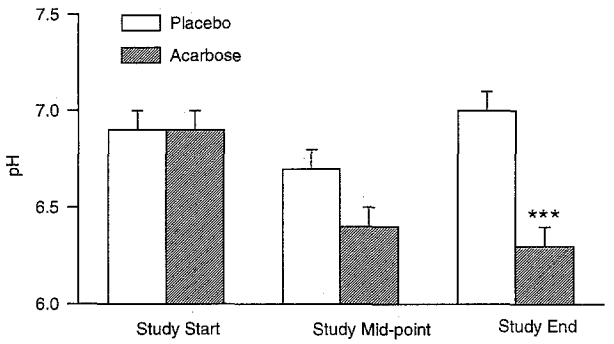


**Fig 3. Fecal pH in placebo- and acarbose-treated subjects. Data are presented as the mean pH with bars designating SE in all subjects at the time points shown. ***$P < .001$. Differences between start and midpoint were also significant ($P < .05$) when calculated for the change in each subject individually.**

fecal concentration per gram dry weight at any of the study periods (Table 1). However, total outputs of butyric acid (Fig 5) and acetic and propionic acids (data not shown) were significantly greater in acarbose-treated subjects than in the placebo group at study end.

Analysis of the distribution of SCFA in the feces calculated as a percent of total SCFA is shown in Table 2. The percent of SCFA present as butyrate in acarbose-treated subjects was significantly greater than in controls at study end.

*Fecal Digoxin-Metabolizing Bacteria and DAG Content*

Digoxin-metabolizing bacteria were cultured from the feces of the majority of subjects in the placebo- and acarbose-treated groups throughout the study. The number

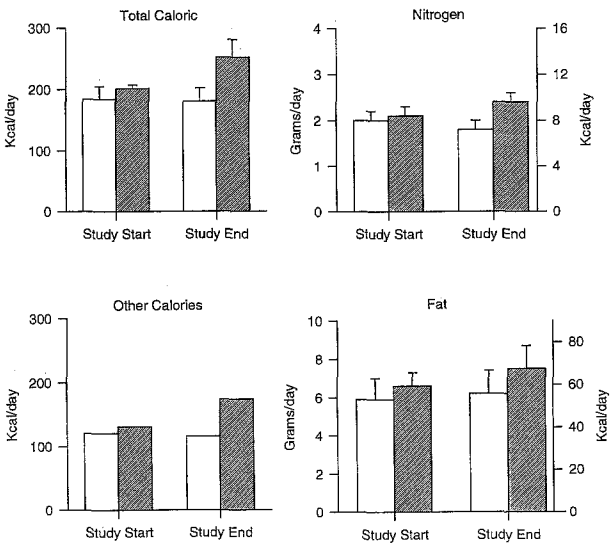


**Fig 4. Fecal output of energy, fat, and nitrogen in placebo- (□) and acarbose-treated (▨) subjects. Data are presented as the mean output with bars designating SE in all subjects at the time points shown. There were no significant differences ($P > .05$) between the 2 groups at any time point or when calculated for the change in each subject individually. Other energy, energy not accounted for by fecal output of fat and nitrogen.**

**Table 1. Effect of Acarbose on Fecal SCFA Concentrations**

| Parameter | Placebo*† | Acarbose* |
|---|---|---|
| Total SCFA | | |
| Start | 319 ± 41 | 310 ± 32‡ |
| Midpoint | 504 ± 93 | 457 ± 48§ |
| End | 452 ± 190 | 435 ± 35‖ |
| Acetic acid | | |
| Start | 198 ± 28 | 227 ± 40‡ |
| Midpoint | 267 ± 52 | 274 ± 27§ |
| End | 253 ± 103 | 263 ± 21‖ |
| Butyric acid | | |
| Start | 53.2 ± 9 | 57.4 ± 8‡ |
| Midpoint | 126.0 ± 42 | 114.0 ± 18§ |
| End | 75.9 ± 38 | 75.6 ± 10‖ |
| Propionic acid | | |
| Start | 42.5 ± 7 | 58.6 ± 10‡ |
| Midpoint | 62.4 ± 12 | 65.5 ± 11§ |
| End | 66.9 ± 32 | 64.2 ± 8‖ |

NOTE. Data are the mean ± SE for the number of subjects in whom complete data were available. No significant differences between total or individual SCFA were found when comparing placebo- and acarbose-treated patients.

*mmol/g dry weight.

†n = 15.

‡n = 30.

§n = 27.

‖n = 24.

of digoxin-reducing bacteria was generally stable in both groups. A change in the concentration of these bacteria that was considered significant (a concentration increase or decrease >3 log/g feces) was only detected in approximately 10% of subjects in either group. There was a slight reduction in the concentration of organisms at the midpoint in acarbose-treated subjects (Table 3) that may have been in part due to increased fecal water content.

Fecal DAG production did not change significantly throughout the study in either subject group (Table 4).

*Breath Hydrogen Analysis*

Breath hydrogen output calculated as total output or as total minus baseline output did not differ at the start of the study between placebo- and acarbose-treated subjects (Fig 6). However, breath hydrogen output was significantly greater in acarbose-treated subjects versus placebo-treated

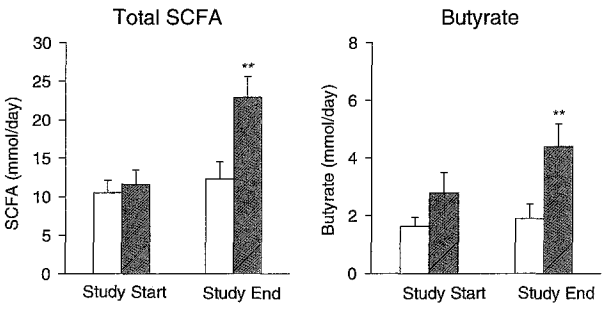


**Fig 5. Fecal output of total SCFA and butyric acid in placebo (□) and acarbose-treated (▨) subjects. Data are presented as the mean output with bars designating SE in all subjects at the time points shown. **$P < .01$.**

**Table 2. SCFA Distribution in Placebo- and Acarbose-Treated Subjects**

| | Placebo | | | | Acarbose | | | |
|---|---|---|---|---|---|---|---|---|
| SCFA | No. | Start (%) | Midpoint (%) | End (%) | No. | Start (%) | Midpoint (%) | End (%) |
| Acetic | 15 | 59.4 | 54.8 | 59.9 | 23 | 54.6 | 61.1‡ | 63.2§ |
| Propionic | 15 | 12.8 | 12.3 | 14.9 | 24 | 17.1 | 12.6‡ | 13.6 |
| Butyric | 15 | 15.0 | 19.9 | 12.6 | 23 | 17.7 | 21.1‡ | 17.9† |
| Isobutyric | 15 | 3.3 | 3.0 | 3.3 | 24 | 2.8 | 1.4‡ | 1.7*§ |
| Isovaleric | 15 | 3.7 | 3.4 | 4.0 | 23 | 4.4 | 1.9*‖ | 2.2*‡ |
| Valeric | 15 | 2.6 | 2.4 | 2.5 | 23 | 2.8 | 2.1‡ | 1.8§ |
| Other | 15 | 3.2 | 4.2 | 2.8 | 23 | 0.6 | 0 | 0 |

NOTE. Results are the mean percent in the number of subjects for whom complete data were available.

*$P < .05$, acarbose *v* placebo.

†$P < .01$, acarbose *v* placebo.

‡$P < .05$, acarbose start *v* midpoint.

§$P < .05$, acarbose start *v* end.

‖$P < .001$, acarbose start *v* midpoint.

subjects at both the midpoint and end point. No differences in breath hydrogen output were found between the midpoint and end point in acarbose-treated subjects (Fig 6).

*Changes in Rectal Crypt Proliferative Kinetics*

The results of studies of rectal crypt proliferative indices are shown in Table 5. Crypt cell number did not differ in the two groups of subjects at study start or study end, indicating that there was no evidence of crypt cell hyperplasia. Furthermore, the number of labeled cells per crypt did not differ significantly at the two study periods, but there was a modest reduction in placebo-treated subjects from 4.5 labeled cells per crypt at study start to 3.46 at study end ($P = .09$). The labeling index in the whole crypt decreased significantly in placebo-treated subjects from a mean of 9.3 ± 0.8 to 6.9 ± 0.6 ($P < .04$). There was no difference in acarbose-treated subjects.

To further explore the effect of changes in colonic luminal contents on proliferative kinetics, correlations were determined between changes in fecal composition and the whole crypt cell labeling index, the Φ h labeling index, and the compartment 4 labeling index (Table 6). No significant correlations were detected between proliferative indices and breath hydrogen output, fecal DAG concentration, fecal pH, or fecal propionic acid concentration or output. However, combining the data from both placebo- and acarbose-treated subjects, multiple negative correlations between total fecal SCFA and rectal crypt labeling indices were found. Total SCFA concentrations and output showed

**Table 3. Fecal Digoxin-Metabolizing Bacteria in Placebo- and Acarbose-Treated Subjects (log organisms/g)**

| Time | Placebo | Acarbose | *P* |
|---|---|---|---|
| Start | 3.3 ± 0.7 (n = 15) | 2.8 ± 0.5 (n = 30) | .55 |
| Midpoint | 3.7 ± 0.6 (n = 15) | 2.1 ± 0.4 (n = 27) | .04 |
| End | 3.7 ± 0.5 (n = 15) | 2.3 ± 0.5 (n = 24) | .07 |

NOTE. Results are the mean ± SE log of the number of organisms per gram wet feces in the number of subjects fromwhom specimens were available. Total fecal output of organisms did not differ in the 2 groups.

**Table 4. Fecal DAG in Placebo- and Acarbose-Treated Subjects**

| DAG | Placebo | Acarbose |
|---|---|---|
| Concentration (mmol/fecal g dry weight) | | |
| Start | 1.05 ± 0.14 (n = 15) | 1.14 ± 0.14 (n = 29) |
| End | 0.98 ± 0.2 (n = 15) | 1.31 ± 0.22 (n = 24) |
| Total output (mmol/d) | | |
| Start | 35.1 ± 5.3 (n = 15) | 45.3 ± 7.6 (n = 29) |
| End | 40.6 ± 13.1 (n = 15) | 65.5 ± 11.1 (n = 24) |

NOTE. Data are the mean ± SE in the number of subjects for whom complete data were avilable. No significant differences in concentration and total output were found.

a negative correlation with labeling of the upper crypt (compartment 4 and Φ h). Furthermore, fecal butyrate content and concentration also were negatively correlated with labeling indices in these regions of the upper rectal crypt (Table 6). There were no correlations between fecal output of other SCFA and the proliferative indices measured.

## DISCUSSION

Acarbose is one of a group of inhibitors of intestinal α-glycosidase enzymes that recently have been introduced to treat patients with type I and type II diabetes mellitus.[3] These agents extend the dietary control of the disease. Our study demonstrates that acarbose, at doses between 50 and 300 mg three times daily with each meal, increases fecal wet weight but does not lead to excessive fecal excretion of energy, fat, protein, or carbohydrate (the last determined as "other components" in the stool). These data indicate that acarbose administration does not lead to fecal loss of the major macronutrients. This is supported by the results of a previous short-term study of another glycosidase inhibitor, nojirimycin,[7] and by the clinical observation that neither our study patients nor large numbers of diabetic patients treated with acarbose lost weight.[18,19]

The treatment was well tolerated by most subjects, and only six of 45 individuals withdrew from this 1-year study. Five withdrew because of unacceptable side effects, principally excessive flatus and/or abdominal cramps, resulting from acarbose administration. An evaluation of subjects who withdrew and their symptoms indicated that these

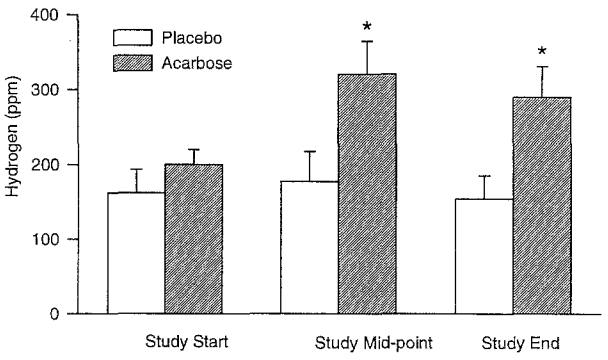


**Fig 6. Breath hydrogen output in placebo- and acarbose-treated subjects. Data are presented as the mean output of hydrogen with bars designating SE in all subjects at the time points shown. *$P < .05$.**

**Table 5. Proliferative Kinetics in Placebo- and Acarbose-Treated Subjects**

| Group | Columns Counted (no.) | Cells per Crypt (no.) | Labeled Cells per Crypt (%) | Total Crypt Cell Labeling Index (%) | Compartment 4 Labeling Index | Φ h ($\times 10^{-2}$) |
|---|---|---|---|---|---|---|
| Placebo | | | | | | |
| Start | 391 | 49.0 ± 1.6 | 4.50 ± 0.4 | 9.3 ± 0.8 | 0.183 | 0.039 |
| End | 244 | 49.6 ± 3.0 | 3.46 ± 0.4 | 6.9 ± 0.6* | 0.225 | 0.057 |
| Acarbose | | | | | | |
| Start | 697 | 49.1 ± 1.2 | 3.96 ± 0.2 | 8.3 ± 0.5 | 0.213 | 0.050 |
| End | 357 | 51.1 ± 1.9 | 3.91 ± 0.5 | 7.6 ± 0.8 | 0.313 | 0.068 |

NOTE. Data are the mean ± SE in the number of crypt columns counted in subjects for whom satisfactory specimens were obtained.
*Significantly different ($P < .05$) from study start. Other data show no differences between start and end of study.

were dose-related, the majority occurring at the highest acarbose doses administered (200 to 300 mg three times daily). This resulted in part because the study was designed as a rapid forced-titration to a dose that produced side effects. In clinical use, acarbose is well tolerated when introduced at low doses and is gradually increased as abdominal symptoms decrease.

The abdominal symptoms result from bacterial breakdown of carbohydrates that pass into the colon because of acarbose-induced small intestine digestive impairment. Bacterial enzymes produce osmotically active metabolites and gases from carbohydrate substrates. In our study, excess postprandial breath hydrogen output indicates such increased bacterial metabolism. Furthermore, there was a modest but significant increase in fecal water content in acarbose-treated subjects.

A further consequence of increased bacterial metabolism of carbohydrate with acarbose in our study was a decrease in pH from approximately 7.0 at study start to 6.3 at study end. This was accompanied and presumably produced mainly by a twofold increase in fecal output of SCFA. A decrease in pH may greatly alter the absorption of calcium,[20] precipitate intraluminal unconjugated bile acids,[21] and reduce the absorption of fatty acids by reducing ionization.[22] Soluble bile acids and fatty acids have been incriminated as damaging the colon mucosa,[23,24] perhaps resulting in pathologic changes and proliferation. Thus, a reduced pH via decreasing the absorption of these compounds may be beneficial to the colon. However, reducing the colonic pH from 6.6 to 6.3 by administration of sodium sulfite did not affect proliferation kinetics in a short-term study in the rat.[25]

**Table 6. Correlations between Fecal SCFA and Rectal Crypt Labeling Indices**

| | | Total Crypt Cell Labeling Index | | Compartment 4 Labeling Index | | Φ h Labeling Index | |
|---|---|---|---|---|---|---|---|
| Parameter | No. | *r* | *P* | *r* | *P* | *r* | *P* |
| Total SCFA | | | | | | | |
| Start + end | | | | | | | |
| Concentration | 60 | −.151 | NS | −.211 | .1 | −.240 | .07 |
| Output | 60 | −.167 | NS | −.241 | .06 | −.257 | .05 |
| Start | | | | | | | |
| Output | 35 | −.094 | NS | −.339 | .05 | −.361 | .03 |
| Butyric acid | | | | | | | |
| Start + end | | | | | | | |
| Concentration | 60 | −.193 | NS | −.243 | .05 | −.267 | .04 |
| Output | 60 | −.231 | .08 | −.265 | .02 | −.293 | .02 |
| Start | | | | | | | |
| Output | 38 | −.179 | NS | −.300 | .06 | −.326 | .05 |

NOTE. Only correlations of $P < .1$ between SCFA and labeling indices are presented. Slopes of correlations (*r*) are shown for the number of subjects for whom data were available.
Abbreviation: NS, relationship not significant at $P > .1$.

Acarbose administration increased fecal excretion of total SCFA twofold specifically due to acetic, butyric, and propionic acids. Increased concentrations of colonic SCFA are believed to be beneficial to colonic health,[26,27] and butyrate is a primary metabolic fuel for colonocytes.[26] SCFA are trophic to the colonic epithelium if the rat bowel is deprived of digestible substrate,[28] stimulating proliferation in the basal portion of the crypt. In cell culture systems, sodium butyrate reduces proliferation in neoplastic or transformed colon cancer cell lines,[26] as well as inducing differentiation.[30] Thus, SCFA may increase cell production in the absence of other metabolic substrates, but reduces abnormal proliferation in more neoplastic tissue.

Changes in colonic epithelial cell proliferation have been used extensively in the past two decades to identify individuals at risk for developing colon neoplasia[31] and to identify putative chemopreventive agents.[32] Several lines of evidence support this concept. Increased proliferation results in a longer exposure of cellular DNA to environmental carcinogenic agents,[33] ie, mitogenesis leading to mutagenesis.[34] Furthermore, progression of the normal flat "mucosa" to adenomatous polyps and then to colon cancer is accompanied by a progressive increase in the number of proliferating cells near or in the surface epithelium.[35] Agents that alter colonic proliferation toward normality are thought to have chemopreventive properties for colon neoplasia.[32]

The present study evaluated the effect of acarbose or placebo on cell proliferation in rectal crypts. Acarbose administration was not associated with significant changes in crypt cell number, labeling index in the whole crypt, or distribution of labeled cells into the upper crypt. Placebo-treated subjects showed a significant decrease in the whole crypt labeling index from 9.3% to 6.9% between the beginning and end of the study. However, there were no differences between acarbose and placebo subjects at study end, nor was there a change in the number of labeled cells in the upper crypt in subjects who received placebo. These

data indicate that acarbose did not have a harmful effect on proliferation kinetics.

We also analyzed correlations between cell proliferation and total and individual SCFA fecal concentration and output. This was possible because we had a large number of observations for the output of total SCFA and individual SCFA with widely differing concentrations between controls and acarbose-treated subjects. There was an inverse correlation between the output of both total SCFA and butyric acid and the rectal total crypt labeling index and the labeling indices of the upper crypt. Thus, the higher the fecal SCFA or butyric acid output, the lower the rectal crypt labeling indices. Fiber ingestion generally is believed to reduce the risk of colon cancer through formation of SCFA. In animal studies, the effects of fiber administration and butyrate on colonic proliferation have been controversial.[36,37] The present human studies support a putative protective effect of SCFA on these biomarkers of colorectal neoplasia.

Colonic carbohydrate metabolism resulting from acarbose could alter colonic bacterial flora and have deleterious consequences. To explore this, we studied changes that involve three bacterial parameters. Hydrogen production and breath hydrogen excretion results from carbohydrate metabolism by anaerobic bacteria.[38] Hydrogen output increased in acarbose-fed subjects during the study even when identical test meals plus acarbose were administered. These results suggest that colonic bacterial flora had adjusted to acarbose-induced chronic carbohydrate malabsorption to increase carbohydrate metabolism. This increase in breath hydrogen was observed by the midpoint of the study and did not change thereafter. The increase occurred despite a decrease in pH, which has been shown to decrease colonic hydrogen production.[39] Colonic symptoms including meteorism and abdominal distension were ameliorated or better tolerated with time on acarbose feeding in this and previous studies.[40] In analogous studies of colonic adaptation to feeding lactose[41] or lactulose,[42] evidence for reduced stool frequency and breath hydrogen was obtained after initiation of carbohydrate feeding. This was accompanied by an increase in fecal β-glycosidase activity and a decrease in bowel fluid pH. These metabolic changes can salvage energy from carbohydrate and may ameliorate colonic symptoms. The feeding of lactulose also decreases the concentration of bile acids[43] and may even reduce cholesterol lithogenicity in bile.[44] By analogy, such beneficial effects also might accompany acarbose feeding, but no studies were performed to evaluate this possibility.

Next, we studied fecal concentrations of digoxin-metabolizing bacteria as a model of how changes in the colonic luminal environment can affect drug metabolism. Only a proportion of individuals harbor such bacteria,[45] which can chronically metabolize digoxin into inactive compounds.[46] These individuals require larger doses of oral digoxin to achieve and maintain therapeutic digitalis levels in the blood.[47] When digoxin-metabolizing bacteria are abruptly eliminated by the use of antibiotics, serum digoxin can increase to potentially toxic levels.[45] Since a similar number of individuals gained and lost digoxin-metabolizing bacteria and since the fecal output of these organisms did not differ in the two groups of subjects, acarbose is unlikely to affect this important bacterial species.

Specific colonic organisms are able to form DAG from phosphatidyl choline,[48] and the concentration of fecal DAG differs widely between individuals. DAG can be absorbed by the colonic mucosa[49] and may stimulate protein kinase and colonic proliferation.[50] Since high rates of proliferation are seen in populations at risk for colon neoplasia and since fecal DAG species can be modified by the diet,[51] we studied fecal excretion of DAG throughout the study. No differences in either DAG concentration or output during administration of acarbose was observed, again suggesting little effect on another species of colonic metabolizing bacteria.

In conclusion, this study demonstrates that ingestion of acarbose, a potent α-glycosidase inhibitor used for the treatment of diabetes mellitus, results in passage of some dietary carbohydrate into the colon, but no significant loss of excess energy into the feces. Utilization of carbohydrate by colonic bacteria induces the production of hydrogen and SCFA—mainly acetate and butyrate. Long-term administration of acarbose is accompanied by an increase in colonic bacterial hydrogen production but no change in two other bacterial species, digoxin-metabolizing bacteria, or bacterial production of DAG. Acarbose feeding did not result in changes in rectal epithelial cell proliferation in subjects for whom complete data were available. However, evidence for a correlation of reduced labeling of upper-crypt epithelial cells with changing fecal SCFA and butyrate levels was obtained. This suggests that the increased colonic SCFA resulting from acarbose may induce beneficial and putative antineoplastic effects in the colon. Despite frequent colonic symptoms such as excessive flatus in many patients taking acarbose in full doses, this study provides evidence for the absence of deleterious consequences of the drug.

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## REFERENCES

1. Clissold SP, Edwards C: Acarbose. A preliminary review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential. Drugs 35:214-243, 1988

2. Rodier M, Richard JL, Monnier L, et al: Effect of long term acarbose (Bay g 5421) therapy on metabolic control of non insulin dependent (type II) diabetes mellitus. Diabete Metab 14:12-14, 1988

3. Chiasson JL, Josse RG, Hunt JA, et al: The efficacy of acarbose in the treatment of patients with non–insulin-dependent diabetes mellitus. Ann Intern Med 121:928-935, 1994

4. Jenkins DJA, Taylor RH, Goff DV, et al: Scope and specificity of acarbose in slowing carbohydrate absorption in man. Diabetes 30:951-954, 1981

5. Caspary WF, Lembocke B, Creutzfeldt W: Delaying of

carbohydrate absorption by glucosidase inhibitors, in Creutzfeldt W, Folsch U (eds): Delaying Absorption as a Therapeutic Principle in Metabolic Disease. Stuttgart, Germany, Thieme, 1983, pp 87-95

6. Kotler DP, Holt PR, Rosensweig NS: Modification of the breath hydrogen test: Increased sensitivity for the detection of carbohydrate malabsorption. J Lab Clin Med 100:795-805, 1982

7. Holt PR, Thea D, Yang MY, et al: Intestinal and metabolic responses to an α-glucosidase inhibitor in normal volunteers. Metabolism 37:1163-1170, 1988

8. Steinbach G, Reddy BS, Lupton J, et al: Effect of calcium supplementation on rectal epithelial hyperproliferation in intestinal bypass subjects. Gastroenterology 106:1162-1167, 1994

9. Folch J, Leek M, Sloane-Stanley GH: A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497-509, 1975

10. Ferrari A: Nitrogen determination by a continuous digestion and analysis system. Ann NY Acad Sci 87:792-800, 1960

11. Benedict FG, Fox EL: A method for the determination of the energy values of food and excreta. J Biol Chem 67:783-787, 1925

12. Butler VP Jr, Tse-Eng D, Lindenbaum J, et al: The development and application of a radioimmunoassay for dihydrodigoxin, a digoxin metabolite. J Pharmacol Exp Ther 221:123-131, 1982

13. Dobkin JF, Saha JR, Butler VP Jr, et al: Digoxin-inactivating bacteria: Identification in human gut flora. Science 220:325-327, 1983

14. Steinbach G, Morotomi M, Nomoto K, et al: Calcium reduces the increased fecal 1,2-*sn*- content in intestinal bypass patients: A mechanism for altering colonic hyperproliferation. Cancer Res 54:1194-1197, 1994

15. Steinbach G, Lupton JR, Reddy BS, et al: Effect of calcium supplementation on rectal epithelial hyperproliferation in intestinal bypass patients. Gastroenterology 106:1162-1167, 1994

16. Lipkin M, Blattner WA, Fraumeni JR Jr, et al: Tritiated thymidine labeling distribution in the identification of hereditary predisposition to colon cancer. Cancer Res 43:1899-1904, 1983

17. Satterthwaite FW: An approximate distribution of estimates of variance components. Biometrics Bull 2:110-114, 1946

18. Coniff RF, Shapiro JA, Seaton TB, et al: Multicenter, placebo-controlled trial comparing acarbose (BAY g 5421) with placebo, tolbutamide and tolbutamide-plus-acarbose in non–insulin-dependent diabetes mellitus. Am J Med 98:443-451, 1995

19. Coniff RF, Shapiro JA, Seaton TB: Long-term safety and efficacy of acarbose in the treatment of obese subjects with noninsulin dependent diabetes mellitus. Ann Intern Med 154:2442-2448, 1994

20. Newmark HL, Lupton JR: Determinants and consequences of colonic luminal pH: Implications for colon cancer. Nutr Cancer 14:161-173, 1990

21. Fini A, Roda A: Chemical properties of bile acids. IV. Acidity constants of glycine-conjugated bile acids. J Lipid Res 28:755-759, 1987

22. Newmark HL, Wargovich MS, Bruce WR: Colon cancer and dietary fat, phosphate and calcium. A hypothesis. J Natl Cancer Inst 72:1323-1325, 1984

23. Lapré JA, Termont DSML, Groen AK, et al: Lytic effects of mixed micelles of fatty acids and bile acids. Am J Physiol 263:G333-G337, 1992

24. Lee DYK, Lupton JR, Aukema HM, et al: Dietary fat and fiber alter rat colonic mucosal lipid mediators and cell proliferation. J Nutr 123:1808-1817, 1993

25. Kashtan H, Gregoire R, Bruce WR, et al: Effects of sodium sulfate on fecal pH and proliferation of colonic mucosa in patients at high risk for colon cancer. J Natl Cancer Inst 82:950-952, 1990

26. Roediger WEW: Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut 21:793-798, 1980

27. Scheppach W: Effects of short chain fatty acids on gut morphology and function. Gut 35:535-538, 1994 (suppl 1)

28. Roediger WEW: The starved colon—diminished mucosal nutrition, diminished absorption, and colitis. Dis Colon Rectum 33:858-862, 1990

29. Sakata T: Effects of short-chain fatty acids on epithelial cell proliferation and mucus release in the intestine, in Roche AF (ed): Short-Chain Fatty Acids: Metabolism and Clinical Importance. Report of the Tenth Ross Conference on Medical Research. Columbia, OH, Ross Laboratories, 1991, pp 63-67

30. Newmark HL, Young W: Butyrate and phenylacetate as differentiating agents: Practical problems and opportunities. J Cell Biochem [Suppl] 22:247-253, 1995

31. Lipkin M: Biomarkers of increased susceptibility to gastrointestinal cancer: New application to studies of cancer prevention in human subjects. Cancer Res 48:235-245, 1988

32. Lipkin M, Levin B, Kim Y, et al: Intermediate biomarkers of precancer and their applications in chemoprevention. J Cell Biochem [Suppl] 16G:1-122, 1992

33. Cohen SM, Purtilo DT, Ellwein LB: Pivotal role of increased cell proliferation in human carcinogenesis. Mod Pathol 4:371-382, 1991

34. Ames BN, Gold LS: Endogenous mutagens and the causes of aging and cancer. Mutat Res 250:3-16, 1991

35. Lipkin M: Phase 1 and phase 2 proliferative lesions of colonic epithelial cells in diseases leading to colonic cancer. Cancer 34:878-888, 1974

36. Boffa LC, Lupton JR, Mariani MR, et al: Modulation of colonic epithelial cell proliferation, histone acetylation, and luminal short chain fatty acids by variation of dietary fiber (wheat bran) in rats. Cancer Res 52:5906-5912, 1992

37. Lupton JR: Butyrate and colonic cytokinetics: Differences between in vitro and in vivo studies. Eur J Cancer Prev 4:373-378, 1995

38. Levitt MD: Production and excretion of hydrogen gas in man. N Engl J Med 281:122-127, 1969

39. Perman JA, Modler S, Olson A: Role of pH in production of hydrogen from carbohydrates by colonic bacterial flora. J Clin Invest 67:643-650, 1981

40. Hoffman J, Spengler M: Efficacy of 24-week monotherapy with acarbose, glibenclamide, or placebo in NIDDM patients: The Essen Study. Diabetes Care 17:561-566, 1994

41. Scrimshaw NS, Murray EB: Adaptation of lactose-maldigesting individuals to milk and milk products. Am J Clin Nutr 48:1118-1119, 1988 (suppl)

42. Flourie B, Briet F, Florent C, et al: Can diarrhea induced by lactulose be reduced by prolonged ingestion of lactulose? Am J Clin Nutr 58:369-375, 1993

43. Van Berge Henegouwen GP, Van Der Werf SDJ, Ruben ATH: Effect of long term lactulose ingestion on secondary bile salt metabolism in man: Potential protective effect of lactulose in colonic carcinogenesis. Gut 28:675-680, 1987

44. Thornton JR, Heaton KW: Do colonic bacteria contribute to cholesterol gall-stone formation? Effects of lactulose on bile. Br Med J 282:1018-1020, 1981

45. Lindenbaum J, Rund DG, Butler VP, et al: Inactivation of digoxin by the gut flora: Reversal by antibiotic therapy. N Engl J Med 305:789-794, 1981

46. Lindenbaum J, Tse-Eng D, Butler VP Jr, et al: Urinary excretion of reduced metabolites of digoxin. Am J Med 71:67-74, 1981

47. Luchi RJ, Gruber JW: Unusually large digitalis requirements: A study of altered digoxin metabolism. Am J Med 45:322-328, 1968

48. Morotomi M, Guillem JG, Weinstein IB: Production of diacylglycerol, an activator of protein kinase, by human intestinal microflora. Cancer Res 50:3595-3599, 1990

49. Morotomi M, LoGerfo P, Weinstein BI: Fecal excretion, uptake and metabolism by colon mucosa of diacylglycerol in rats. Biochem Biophys Res Comm 181:1028-1034, 1991

50. Weinstein BI: Cancer prevention: Recent progress and future opportunities. Cancer Res 51:5080-5085, 1991

51. Pickering JS, Lupton JR, Chapkin RS: Dietary fat, fiber and carcinogen alter fecal diacylglycerol composition and mass. Cancer Res 55:2293-2298, 1995