

# Fecal Short-Chain Fatty Acid Concentrations and Effect on Ileal Pouch Function

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Random stool samples were obtained from 14 ileal pouch-anal anastomosis (IPAA) patients  $43 \pm 5$  (mean  $\pm$  SEM) months after surgery, and the concentrations of individual short-chain fatty acids (SCFAs) were determined by gas liquid chromatography. Stool frequency was determined from a diary recorded for 15 days prior to stool sampling. The frequency, amplitude, and duration of phasic contractions (PCs) within the pouch following infusion of a physiologic concentration of SCFAs and normal saline randomly into the pouch of six IPAA patients were determined manometrically. The mean total SCFA concentration after IPAA did not differ significantly from normal stools ( $83 \pm 20$  mM after IPAA vs.  $97 \pm 10$  mM for controls;  $P > 0.05$ ). In the IPAA patients, regression analysis demonstrated an *inverse* relationship between stools per day and total SCFA concentration ( $r = 0.73$ ;  $P < 0.001$ ). Moreover, no change in frequency ( $3.0 \pm 0.9$  vs.  $3.2 \pm 0.8$  PCs/30 minutes), amplitude ( $26 \pm 5$  vs.  $25 \pm 4$  mmHg), or duration ( $23 \pm 3$  vs.  $26 \pm 2$  seconds) of PCs was found after SCFA infusion compared with saline control ( $P > 0.1$ ). These findings demonstrate that SCFAs are present in ileal pouch effluent and that stool frequency may be related to fecal SCFA concentration. Also, the normal contractile response of the terminal ileum to SCFAs does not occur in the ileal pouch. [Key words: Short-chain fatty acids; Ileal pouch-anal anastomosis; Inflammatory bowel disease; Ileal pouch; Motility]

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Short-chain fatty acids (SCFAs), the principal organic anions in the colon (predominantly acetic, butyric, and propionic acids), are formed by anaerobic bacterial fermentation of dietary polysaccharides (dietary fiber) not digested in the small bowel.<sup>1</sup> These SCFAs, while having no specific effect on colonic motility, have a trophic effect on the colonic epithelium<sup>2</sup>; colonic mucosal cells re-

ceive most of their nutrition from luminal sources such as SCFAs, without which mucosal cell production falls.<sup>3</sup> Also, the absorptive capacity of the colonic mucosa is decreased when SCFAs are not present in the lumen.<sup>4</sup>

In contrast, SCFAs in the terminal ileum are present in very low concentrations and provide little or no nutritional support to the small bowel epithelium. Also, in contrast to the colon, SCFAs provoke contractile activity when introduced into the lumen of the small bowel.<sup>5</sup> The ability to stimulate small bowel motility has been proposed as one functional mechanism of the ileocecal region; the terminal ileum contracts strongly in response to colonic content rich in SCFAs, thus emptying the small bowel of colonic contents that might reflux past the valve.

After ileal pouch-anal anastomosis (IPAA), the unique situation exists whereby a neorectum (pouch) has been constructed from the terminal ileum. Our aim was to 1) determine the fecal SCFA concentration in patients following IPAA, 2) correlate SCFA concentration with clinical function, and 3) determine the effect of SCFAs on ileal pouch contractile activity.

## MATERIALS AND METHODS

### Subjects

Fourteen patients (six women and eight men, ages  $35 \pm 3$  years; mean  $\pm$  SEM) who had undergone IPAA for chronic ulcerative colitis at least two years earlier were studied. All patients had a J pouch, and  $43 \pm 5$  months (mean  $\pm$  SEM) had passed since closure of their temporary ileostomy. Details of bowel function were obtained from each subject by completing a daily diary of their bowel habits during the 15 days immediately prior to the study. All women of childbearing age had a nega-

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tive pregnancy test. All subjects gave written informed consent of a protocol that had been approved by the Institutional Review Board of the Mayo Clinic.

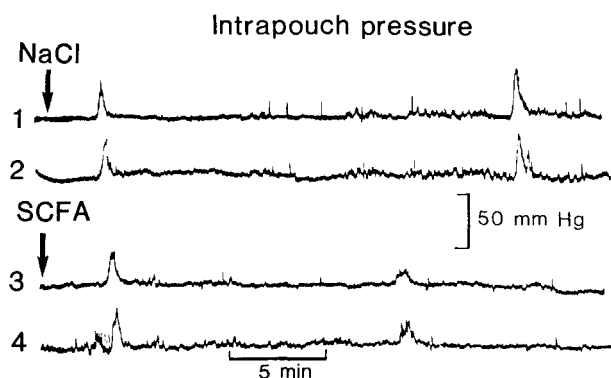
### SCFAs

Random nonfasting stool samples were obtained from 14 patients after IPAA and from six healthy control subjects who did not have an IPAA to determine whether our method of SCFA determination yielded levels consistent with those found in healthy controls by other investigators.<sup>6</sup> The pH of the samples was determined, and then each sample was immediately frozen at  $-20^{\circ}\text{C}$  for later analysis. The concentration of each SCFA including acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids was measured using gas-liquid chromatography. A 1-g sample of feces, to which 3 ml of distilled  $\text{H}_2\text{O}$ , 1 ml of internal standard (2-methylvaleric acid), and  $40\ \mu\text{l}$  of phosphoric acid were added, was steam distilled under vacuum for 15 minutes and then adjusted to a pH of 3.0 with a NaOH solution. Part ( $0.3\ \mu\text{l}$ ) of each sample was analyzed on a Hewlett-Packard Model 5940A gas chromatograph. A 1.8-mm-long  $\times$  2-mm-internal-diameter glass column packed with 10% SP-1200 and 1%  $\text{H}_3\text{PO}_4$ -coated Chromosorb S-AW was used.<sup>7</sup>

### Contractile Activity

After fasting overnight, the pouches of six volunteers were intubated with triple-lumen catheters (Duval Plastics, New South Wales, Australia). The catheters were perfused constantly at 0.1 ml/minute with a low-compliance hydraulic capillary infusion system driven by a pressure head of nitrogen. The infusion system was connected to Statham Gould P23 pressure transducers (Statham Instruments, Inc., Puerto Rico), and the output was displayed on a Honeywell 1600 (Honeywell Technical Instruments, Inc., Denver, CO) multichannel pen recorder with a chart speed of 25 mm/minute. Motility tracings were examined visually for the presence of, amplitude, and duration of discrete phasic contractions, which have been previously described (Fig. 1).

Manometric recordings were obtained for 30 minutes after the perfusion of 50 ml of 154 mM sodium chloride (normal saline) and the infusion of 50 ml of a mixture of the three major SCFAs of colonic contents (acetic 66 percent, propionic 24



**Figure 1.** Intrapouch pressures following instillation of normal saline and physiologic solution of SCFAs.

percent, and butyric 10 percent). The latter mixture consisted of a total concentration of SCFAs of 100 mM; the solution was titrated to 290 mOsm/kg and pH 6.5 with NaCl and NaOH. The SCFAs of the mixture simulate the anions of colonic fluid in most mammalian species.<sup>1, 8</sup> The 50-ml volume is below the threshold volume found to induce pouch contractions.<sup>9</sup> The order of infusion into the pouch of the NaCl and SCFA solutions was randomized. All recordings were done with the patient lying in the left-lateral position.

### Data Analysis

Comparisons of individual and total SCFA concentrations between controls and IPAA patients were done using a Wilcoxon rank sum test. Linear regression analysis was used to correlate stools per day with the logarithm of the individual and total SCFA concentrations. Comparisons of number, amplitude, and duration of high-pressure waves following saline and SCFA infusion were made using a paired Student's *t*-test.

### RESULTS

The stool pH of the ileal pouch patients was  $5.9 \pm 0.1$  (mean  $\pm$  SEM). The mean individual and total SCFA concentrations for ileal pouch effluent and those of stools from control subjects are listed in Table 1. The concentrations of total SCFAs were not significantly different between the ileal pouch patient group and controls. Concentrations of acetic and butyric acid were similar between groups, while the propionic acid concentration was slightly lower in the patient group. Isobutyric, valeric, and isovaleric acids, while present in low concentrations in the control group, were not present in the pouch effluent.

**Table 1.**  
SCFA Concentrations (mM)

	Acetic	Propionic	Butyric	Isobutyric	Valeric	Isovaleric	Total
Controls (n = 6)	50	19	19	2	2	5	97
IPAA (n = 14)	64*	9†	10*	0‡	0‡	0‡	83*

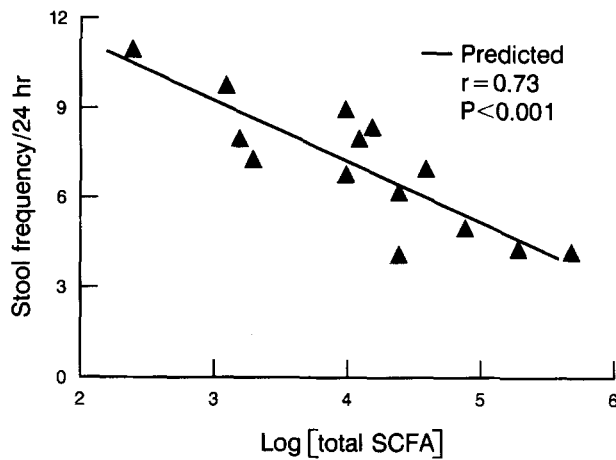
\*  $P > 0.1$

†  $0.05 < P < 0.1$ .

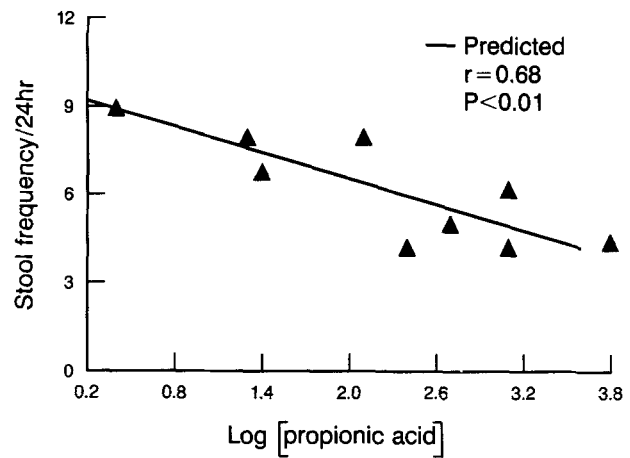
‡  $P < 0.05$ .

Regression analysis demonstrated an inverse relationship between the total SCFA concentration and the total number of stools per day among the patient group (Fig. 2). That is, patients with a greater average number of stools per day had a significantly lower concentration of SCFAs in their stools ( $P < 0.001$ ). In terms of the individual SCFAs, the number of stools per day among the patient group was inversely related to the concentrations of acetic ( $P < 0.001$ ; Fig. 3), propionic ( $P < 0.01$ ; Fig. 4), and butyric ( $P < 0.05$ ; Fig. 5) acids. There was no correlation between the concentrations of isobutyric, valeric, and isovaleric acids and the average number of stools per day ( $P > 0.1$ ).

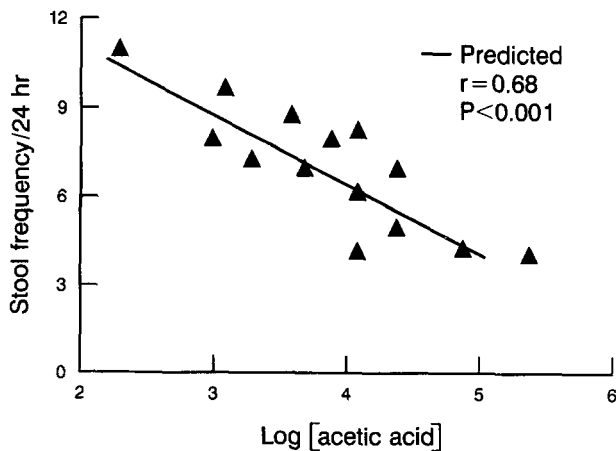
The number of high-pressure waves following an infusion of a physiologic concentration of SCFAs into the ileal pouch did not differ from the number following normal saline infusion ( $P > 0.1$ ; Table 2). Similarly, there was no difference in either the amplitude or the duration of the high-pressure



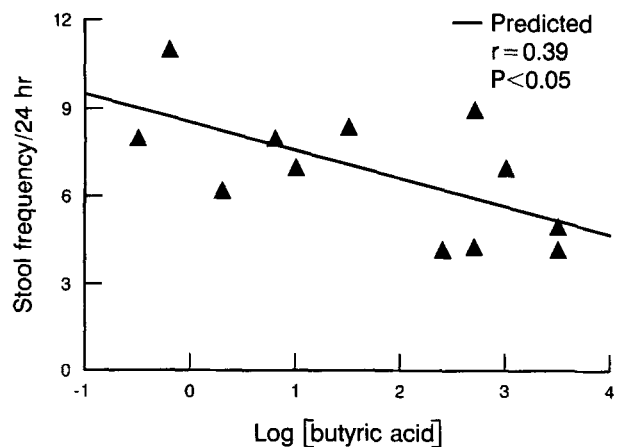
**Figure 2.** Graph of number of stools per day and log of the total SCFA concentration in IPAA stools.



**Figure 4.** Graph of number of stools per day and log of the propionic acid concentration in IPAA stools.



**Figure 3.** Graph of number of stools per day and log of the acetic acid concentration in IPAA stools.



**Figure 5.** Graph of number of stools per day and log of the butyric acid concentration in IPAA stools.

**Table 2.**  
Ileal Pouch Phasic Contractions (Mean + SEM)

Infusate	Number	Amplitude	Duration
Normal saline (n = 6)	3.2 ± 0.8 HPWs/30 min	26 ± 4 mmHg	26 ± 2 sec
SCFAs (n = 6)	3.0 ± 0.8 HPWs/30 min*	26 ± 5 mmHg*	23 ± 2 sec*

HPWs = high-pressure waves.

\*  $P > 0.1$ .

waves following SCFA infusion compared with normal saline ( $P > 0.1$ ; Table 2).

## DISCUSSION

The addition of an ileal pouch to the ileoanal anastomosis procedure was designed to provide a storage reservoir for enteric content in an effort to improve functional results. As might be expected, the pouch becomes colonized with anaerobes and coliforms similar to those found in the rectum.<sup>10,11</sup> We demonstrated in this study that SCFAs, which are the by-products of anaerobic fermentation in the *colon*, are actually present in the effluent of the ileal pouch after IPAA in concentrations similar to those of controls and that the number of stools per day in the IPAA patients is inversely correlated to the concentration of specific SCFAs.

The total fecal concentrations of SCFAs did not differ significantly from controls; nor did the concentrations of the individual SCFAs acetic, propionic, and butyric acids. Isobutyric, valeric, and isovaleric acids, which were present in low concentrations in controls, were not present in the IPAA group. Nasmyth and colleagues<sup>11</sup> found a higher concentration of acetic acid in 11 IPAA patients studied a mean of 12 months after closure of their ileostomy, compared with controls, with similar concentrations of propionic, butyric, and isobutyric acids.<sup>11</sup> As it has been previously demonstrated that function improves over time following ileostomy closure in IPAA patients, the difference in the SCFA concentrations between the two studies may be the result of the continued alteration in flora with the longer interval following surgery in our study.<sup>12</sup>

We found fewer stools per day correlated with a higher concentration of total SCFAs as well as concentrations of the individual SCFAs acetic, propionic, and butyric acids. The extent to which SCFAs contribute to ileal pouch epithelial nutrition is unknown, but ileal pouch villous atrophy has been correlated with a decreased level of butyric acid within the pouch effluent.<sup>11</sup> Also, the effi-

ciency of sodium absorption by the colonic epithelium is decreased markedly in the absence of bacterial SCFAs.<sup>4</sup> Either mucosal atrophy or decreased mucosal absorptive capacity in the pouch might result in the increased stool frequency we noted with decreased fecal SCFA concentrations among the IPAA patients. However, this association remains speculative.

In addition, the normal contractile response of the ileum to infusion of SCFAs was abolished in the ileal pouch. Physiologic concentrations of fecal SCFAs cause the terminal ileum in healthy controls to contract.<sup>5</sup> If this response had been maintained after IPAA, the SCFAs present in the pouch would be expected to induce contractions and thus have an adverse effect on fecal continence. However, we found no increase in pouch contractions following SCFA instillation into the pouch, indicating that, along with other changes that occur following creation of the ileal pouch, this contractile response of the terminal ileum is abolished.

In summary, this study demonstrated that fecal SCFAs in ileal pouch effluent are present in concentrations similar to those of normal stool and that the fecal concentrations of SCFAs in IPAA patients (specifically acetic, propionic, and butyric acids) are inversely related to stool frequency. Moreover, the contractile response of the terminal ileum to SCFA infusion is not present in the ileal pouch. The effect of SCFAs on ileal pouch absorptive capacity and their trophic effects on pouch epithelium as well as their relationship to specific disorders, such as pouchitis, remain to be determined.

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