Calcium and Colorectal Epithelial Cell Proliferation in Ulcerative Colitis

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Abstract
In persons at higher risk for colon cancer (e.g., those with sporadic adenoma or ulcerative colitis), compared to those at lower risk, colonic epithelial cell proliferation kinetics are altered. We have shown previously that calcium supplementation appears to normalize the distribution of proliferating cells without affecting the proliferation rate in the colorectal mucosa of sporadic adenoma patients. In a pilot randomized, double-blind, placebo-controlled, clinical trial conducted concurrently with our previously published sporadic adenoma trial, we tested whether calcium supplementation can also modulate cell proliferation kinetics in patients with ulcerative colitis. Ulcerative colitis patients (n = 31) were randomized to placebo or 2.0 g of supplemental calcium daily. Colorectal epithelial cell proliferation was determined by immunohistochemical detection of proliferating cell nuclear antigen labeling of cells in "non-prep" rectal biopsies taken at randomization and after 2 months treatment. All biopsies were scored by one reviewer. Differences in mean follow-up minus baseline labeling index (LI; the proportion of colon crypt epithelial cells that were labeled) and in the \( \Phi_r \) (proportion of labeled cells that were in the upper 40% of the crypts) were compared with analysis of covariance. Pill-taking adherence was 97%. Biopsy-scoring reliability was high (r = 0.89). The pooled baseline LI and \( \Phi_r \) were 6.3% and 5.6%, respectively. The LI in the calcium group decreased by 0.3% (proportionately, 3%) more than in the placebo group (\( P = 0.91 \)). Similarly, the \( \Phi_r \) in the calcium group decreased by 0.5% (proportionately, 10%) more than in the placebo group (\( P = 0.85 \)). This pilot study does not suggest that 2.0 g of calcium as calcium carbonate daily can substantially normalize either the rate or distribution of proliferating cells over a 2-month period in the colon crypts of patients with ulcerative colitis; a more definitive answer to the question of whether calcium may be effective would require a study with a larger sample size and/or other study design modifications.

Introduction
Colorectal cancer is the second most common cause of cancer deaths in the United States (1). Persons with ulcerative colitis, especially those with pancolitis, are at increased risk of developing colon cancer (2). Dietary calcium supplementation has been consistently found to reduce colon cancer occurrence in experiments in animals (3-9), and the epidemiological literature, although inconsistent, on balance provides additional support for the hypothesis that higher calcium consumption may reduce colon cancer incidence in humans (10).

Cancer administration was found to ameliorate the proliferation abnormalities in the crypts of patients with ulcerative colitis (11). Several studies (12-26) have reported that, compared to patients at low risk for colon cancer, patients with colon cancer (12-21) and patients in every category known to be at higher risk for colon cancer (those with a history of sporadic adenoma (12-15, 17-21), familial polyposis (16, 22), or ulcerative colitis (12, 23, 24); those with a family history of colon cancer (16, 17, 25); and the elderly (13, 26)), on average, exhibit in their normal-appearing mucosa both an increased crypt epithelial cell proliferation rate and an extension of the colon crypt proliferative zone from the lower (basal) 60% of the crypt to include the upper (luminal) 40% of the crypt. In patients with previous colon cancer or sporadic adenomas, these changes also predict adenoma recurrence (27, 28). In large bowel tumors in humans, an upward shift in the proliferative zone is found in colon cancers and adenomas but not in hyperplastic polyps (29). As reviewed elsewhere (30-32), proliferative changes in histologically normal-appearing mucosa have been shown to be a consequence of both cancer-initiating and cancer-promoting agents: proliferative changes both precede and accompany colonic neoplasms in rodents given chemical carcinogens, and a high fat diet produces proliferative changes in both rodents and humans. Evidence from animal experiments and preliminary evidence from human studies strongly suggest that these two proliferation abnormalities, i.e., hyperproliferation and upward shift of the proliferation zone, are reversible biomarkers or precursors for colon neoplasia (30-33). In humans, the two proliferation abnormalities appear to be independent variables (19, 34), and rectal biopsy findings on both measures reflect those throughout the colon (21, 35).

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effects in humans. More recently, there have been three larger controlled trials in humans with sporadic adenoma (45–47). A full-scale \((n = 193)\) trial in sporadic adenoma patients conducted by our group concurrently with the present study found a large, statistically significant, normalizing effect on the proliferative zone (45); however, a second full-scale trial (47) did not, and the third trial (46) did not address this measurement. Possible mechanisms of action of calcium in normalizing colorectal epithelial cell proliferation and reducing the risk of colon cancer include the binding of calcium with bile acids (thought to be promoters of colonic neoplastic change) to form inert soaps (11) and the direct induction by calcium of terminal differentiation of the colonic epithelial cells (30–33).

There have been no reported randomized clinical trials to assess the efficacy of higher calcium consumption in normalizing cell proliferation kinetics in humans with ulcerative colitis. To address this need, we conducted a randomized, double-blind, placebo-controlled clinical trial \((n = 31)\) to determine whether calcium supplementation will reduce the colorectal epithelial cell proliferation rate and normalize the distribution of proliferating cells within colorectal crypts \(i.e.,\) shift the zone of proliferation from one that includes the entire crypt to one that is confined to the lower 60%, or normal proliferative zone, of the crypt in patients with ulcerative colitis.

Materials and Methods

This study was approved by the Committee on Use of Human Subjects in Research of the University of Minnesota. Written informed consent was obtained from each study participant.

Participant Population. Participants were recruited from the patient population attending a private practice gastroenterology group that performs approximately 60% of all colonoscopies in the Minneapolis-St. Paul area. To be eligible for the study, subjects must have been 21–74 years of age, in general good health, and capable of informed consent. They must have had no medical conditions, habits, or medication usage that would interfere with the study as described below. Specific exclusions included: a history of ever having toxic megacolon or other life-threatening complication of ulcerative colitis, hospitalization for treatment of ulcerative colitis within the previous 2 years, Crohn’s disease, calcium supplement use, vegetarian diet, major diet change within the previous 6 months, supplemental daily intake of vitamin D greater than 400 IU or vitamin A greater than 10,000 IU, regular antacid use, bile acid-binding resin use, inability to refrain from aspirin use for 10 days, history of bleeding disorder or current use of anticoagulant medication, history of endocarditis or artificial heart valve, lithotripsy, current use of thiazide diuretics in amounts greater than the equivalent of 50 mg of hydrochlorothiazide daily, immunosuppression, childbearing potential, renal insufficiency, kidney stones within the previous 20 years, hyper- or hypoparathyroidism, hypo- or hyperthyroidism not under control, abnormal serum calcium or creatinine levels at eligibility visit, familial polyposis or Gardner syndrome, intestinal malabsorption syndromes, active liver or pancreatic disease, gastrectomy, bowel resection, enema or laxative dependence, active peptic ulcer disease, active malignancy other than nonmelanoma skin cancer, cardiovascular or pulmonary disease that moderately or severely limited activity, narcotic or alcohol dependence, nondeliberate weight loss of \(\geq 10\%\) in the previous 3 months, and less than 80% compliance to a medication regimen in a 4-week placebo run-in trial.

Clinical Trial Protocol. All age-eligible practice patients who had been diagnosed with ulcerative colitis were identified as potential study participants. All patients passing initial chart screening for eligibility were sent an introductory letter, followed by a brief telephone interview. Potential participants were then scheduled for an eligibility visit, at which time they were interviewed, completed questionnaires, and provided a blood sample. Their diet was assessed with a semiquantitative food frequency questionnaire (48). Medical and pathology records were reviewed. Those eligible entered a 4-week placebo run-in trial. Only participants without significant perceived side effects and who had taken at least 80% of their tablets were eligible for randomization. Compliance for the run-in was assessed by questionnaire, interview, and pill count. Eligible participants then received their baseline biopsy and, if still willing to continue, were randomized (stratified by sex) to one of two groups. All involved in the trial, including study personnel, endoscopists, study participants, and laboratory personnel were blinded to treatment strategy.

Participants \((n = 31)\) were randomly assigned to the two treatment groups: a placebo-control group \((n = 14)\) and a 2.0 g \((n = 17)\) elemental calcium supplementation group. The placebo was free of calcium, magnesium, vitamin D, or chelating agents. As with our calcium and colorectal epithelial cell proliferation in sporadic adenoma patients trial (45), the calcium tablets were calcium carbonate tablets (OcCal; at the time of the study, from Marion Merrill Dow, Inc., Kansas City, MO; now from SmithKline Beecham, Pittsburg, PA) taken twice daily with meals \(i.e.,\) 5.0 g of calcium carbonate daily). Placebo and calcium tablets were identical in size, appearance, and taste. The treatment period was 2 months. At the single follow-up visit 2 months after randomization (baseline), pill-taking adherence was assessed by questionnaire, interview, and pill count. Participants were instructed to remain on their usual diet during the study. Factors hypothesized to be related to colon cell proliferation were assessed at baseline, and several were reassessed at the follow-up (final) visit.

Rectal biopsies were taken from study participants at random assignment to their treatment groups (baseline) and again at 2 months after onset of treatment (follow-up). The rectal biopsy procedure was performed without the participant first taking a laxative, enema, or other bowel-cleansing preparation. Three biopsies that appeared grossly adequate were taken from normal-appearing mucosa of the rectum 10 cm above the level of the anus.

Laboratory Protocol. The laboratory protocol is described in more detail elsewhere (45). Briefly, the biopsies were stretched out flat, lumen side up, on bibulous paper; fixed in 10% ethanol; processed in a tissue processor; embedded in a paraffin block; cut with a microtome so that crypts were longitudinally sectioned from base to lumen (section levels were 3 \(\mu\)m thick and taken 50 \(\mu\)m apart); and mounted on microscope slides. To label proliferating cells, slides were then subjected to immunohistochemical analysis for PCNA3 as summarized: slides were deparaffinized; rinsed; received a blocking agent; slides were then subjected to immunohistochemical analysis for PCNA3 as summarized: slides were deparaffinized; rinsed; received a blocking agent, 5% normal horse serum (diluted in PBS; Vector Laboratories, Burlingame, CA), followed by PC-10 clone PCNA antibody

3 The abbreviations used are: PCNA, proliferating cell nuclear antigen; LI, labeling index; BrdUrd, 5-bromo-2'-deoxyuridine.
Protocol for Scoring Colon Crypt Sections on Slide. The biopsy scoring protocol is described in more detail elsewhere (45). Briefly, colon crypts longitudinally sectioned from base to lumen were analyzed. The total number of cells and number of labeled cells for each crypt scored were counted, and the cell count position of each PCNA-labeled cell within a crypt was recorded. A scorable crypt was defined as an intact crypt extending from the muscularis to the lumen. Only whole crypts were counted. Countable cells were defined as crypt cells in line in a single column of nuclei that extended from base to lumen. An unlabeled cell was defined as a cell with a blue nucleus. A weakly labeled cell (a cell in late G1 or early G2) was defined as a cell with a nucleus that was light brown in color (1-2 intensity). A strongly labeled cell (a cell in S-phase) was defined as a cell with a nucleus that was dark brown in color (3-4 intensity). Each cell was identified as an unlabeled cell, a weakly labeled cell, or a strongly labeled cell. The crypt base center cell was also identified as an unlabeled cell, a weakly labeled cell, or a strongly labeled cell. The crypt base center cell was also identified. The goal was to score a minimum of 10 crypts.

The number of labeled cells in the crypt was determined by direct count of all labeled cells in the crypt. The position or height of a labeled cell was assigned by cell count number from the center cell of the crypt base.

One slide reader scored slides throughout the study. Slides from a 10% sample of biopsies scored by the reader were resubmitted in a blinded manner to reread to determine intrarater reliability.

Statistical Analysis. Treatment groups were assessed for comparability of characteristics at baseline and at final follow-up by \( \chi^2 \) test for categorical variables and by analysis of covariance for continuous variables; sex was included as a covariate. Reader reliability was analyzed using intraclass correlation coefficients.

The overall cell proliferation rate, the LI, was calculated for each biopsy by dividing the total number of labeled cells counted (LC) by the total number of cells counted (TC) from all scored crypts from the biopsy and multiplying by 100% (100% \( \times \text{LC/TC} \); Ref. 18). Crypts were subdivided horizontally by cell count height into five equal-sized compartments (18). A measure of the distribution of proliferating cells in the crypt, the proportion of proliferating cells that were in the upper (luminal) 40% of the crypt (\( \text{LC}_{1-4} \)), was calculated on each biopsy by dividing the number of labeled cells counted in the upper 40% of the crypt (\( \text{LC}_{1-4} \)) by the total number of labeled cells counted (LC) and multiplying by 100% (\( \text{LI} = 100\% \times \frac{\text{LC}_{1-4}}{\text{LC}} \); Ref. 16). Each of these measures was calculated separately using both the 3-4" labeled cells (strongly labeled cells) as the labeled cells and the 1-4" labeled cells (all labeled cells) as the labeled cells.

Mean colorectal epithelial cell proliferation parameters were calculated for each treatment group at baseline and at the 2-month follow-up. Changes in proliferation parameters over time were computed within each treatment group as person-specific follow-up minus baseline differences, and then mean changes were calculated and compared using analysis of covariance (with sex as a covariate). Analyses were performed on both the raw data and on the data transformed to improve normality as described previously (45). However, because the results of the analyses did not differ substantially, for simplicity, only the analyses on the raw data are presented herein.

In addition, the baseline cell proliferation data from both treatment groups were combined and compared with those from the sporadic adenoma patients accrued during the same time period in our concurrently conducted calcium and colorectal epithelial cell proliferation trial (45). Mean age- and sex-adjusted cell proliferation values were computed and compared using analysis of covariance.

Results

All patients were white, none had a history of having an adenoma, and none had a history of a first-degree relative with colon cancer. Treatment groups did not differ significantly on any other characteristics measured at baseline (for examples, see Table 1) or at the end of the study.

Biopsies that were scorable were obtained on 28 participants at baseline and 24 at 2 months; scorable biopsies were obtained at both baseline and 2 months on 21 participants. A total of 85% of biopsies had one or more scorable crypts and adequate labeling, and 84% had eight or more scorable crypts and adequate labeling. Treatment groups did not differ significantly at baseline on the mean number of crypts scored per participant, on crypt characteristics, or on cell proliferation characteristics (Table 2). As shown in Table 3, levels of cell proliferation in the ulcerative colitis patients were higher than those found in the adenoma patients in our concurrent trial in adenoma patients. The S-phase and all-labeled cell measurement alternatives for each proliferative parameter were highly correlated, and analyses using either closely mirrored the other. Consequently, for succinctness, only the more traditionally reported S-phase measures are presented hereafter.

Adherence to visit attendance was 100%. The mean proportion of pills taken in each group was 97% and over 92% of all participants in each group took \( \geq 80\% \) of their pills. There were no treatment or biopsy complications. Intrarater reliability (intraclass correlation coefficient) for biopsy slide scoring was 0.89.

Analyses of clinical trial biopsy results by treatment group are summarized in Table 2. There were no substantial or statistically significant differences in follow-up minus baseline differences between the two treatment groups. For the LI, the absolute follow-up minus baseline differences for the placebo and calcium groups were -1.4% and -1.7%, respectively,
Calcium Trial in Ulcerative Colitis Patients

### Table 1
Selected baseline characteristics of participants (n = 31)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n = 14)</th>
<th>Calcium (n = 17)</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) age (yr)</td>
<td>44 (9.5)</td>
<td>48 (8.3)</td>
<td>0.21</td>
</tr>
<tr>
<td>Men (%)</td>
<td>71</td>
<td>47</td>
<td>0.18</td>
</tr>
<tr>
<td>College graduate (%)</td>
<td>64</td>
<td>47</td>
<td>0.46</td>
</tr>
<tr>
<td>Ulcerative colitis history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) duration (yr)</td>
<td>15 (5.6)</td>
<td>19 (9.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>Dysplasia (%)</td>
<td>7</td>
<td>12</td>
<td>0.84</td>
</tr>
<tr>
<td>On ulcerative colitis medications (%)</td>
<td>71</td>
<td>65</td>
<td>0.36</td>
</tr>
<tr>
<td>Symptoms stable (%)</td>
<td>64</td>
<td>47</td>
<td>0.27</td>
</tr>
<tr>
<td>Mean (SD) No. bowel movements/wk.</td>
<td>11 (6.5)</td>
<td>12 (5.9)</td>
<td>0.40</td>
</tr>
<tr>
<td>Degree of symptoms in the past month (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>64</td>
<td>47</td>
<td>0.27</td>
</tr>
<tr>
<td>Mild</td>
<td>29</td>
<td>41</td>
<td>0.41</td>
</tr>
<tr>
<td>Moderate</td>
<td>7</td>
<td>12</td>
<td>0.62</td>
</tr>
<tr>
<td>Degree of symptoms past 24 hrs. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>71</td>
<td>82</td>
<td>0.75</td>
</tr>
<tr>
<td>Mild</td>
<td>29</td>
<td>18</td>
<td>0.75</td>
</tr>
<tr>
<td>Moderate</td>
<td>0</td>
<td>0</td>
<td>0.75</td>
</tr>
<tr>
<td>Ulcerative colitis history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD) total energy intake (kcal/day)</td>
<td>2154 (546)</td>
<td>2110 (793)</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean (SD) dietary calcium intake (mg/day)</td>
<td>949 (402)</td>
<td>731 (382)</td>
<td>0.13</td>
</tr>
<tr>
<td>Mean (SD) dietary vitamin D intake (IU/day)</td>
<td>304 (256)</td>
<td>288 (214)</td>
<td>0.70</td>
</tr>
<tr>
<td>Mean (SD) total fat intake (% kcal)</td>
<td>30 (2.7)</td>
<td>28 (5.6)</td>
<td>0.33</td>
</tr>
<tr>
<td>Mean (SD) dietary fiber intake (g/day)</td>
<td>19 (7.2)</td>
<td>21 (10.6)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*a Calcium, 2.0 g of elemental calcium per day delivered as two 1.25-g calcium carbonate tablets taken twice daily.

*b By analysis of covariance for continuous variables (adjusted for sex) and by extended $\chi^2$ for categorical variables.

### Table 2
Summary of clinical trial rectal biopsy results: placebo versus calcium

<table>
<thead>
<tr>
<th>Biopsy measurement</th>
<th>Placebo (Baseline n = 14, Mean (SD))</th>
<th>Placebo (FU – BL&lt;sup&gt;a&lt;/sup&gt; n = 9, Mean (SD))</th>
<th>Calcium (Baseline n = 14, Mean (SD))</th>
<th>Calcium (FU – BL&lt;sup&gt;a&lt;/sup&gt; n = 12, Mean (SD))</th>
<th>p&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of crypts</td>
<td>9.5 (1.0)</td>
<td>0.1 (1.1)</td>
<td>9.3 (2.1)</td>
<td>-1.3 (4.6)</td>
<td>0.71</td>
</tr>
<tr>
<td>Total No. of cells/crypt</td>
<td>121.5 (13.1)</td>
<td>2.3 (24.5)</td>
<td>131.3 (20.3)</td>
<td>-6.6 (35.7)</td>
<td>0.11</td>
</tr>
<tr>
<td>No. of labeled cells/crypt&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.5 (5.1)</td>
<td>-1.4 (6.9)</td>
<td>8.9 (4.4)</td>
<td>-2.1 (4.9)</td>
<td>0.33</td>
</tr>
<tr>
<td>LI&lt;sup&gt;d&lt;/sup&gt; (%)</td>
<td>6.0 (3.7)</td>
<td>-1.4 (4.6)</td>
<td>6.6 (2.6)</td>
<td>-1.7 (3.3)</td>
<td>0.47</td>
</tr>
<tr>
<td>Overall</td>
<td>6.0 (3.7)</td>
<td>-1.4 (4.6)</td>
<td>6.6 (2.6)</td>
<td>-1.7 (3.3)</td>
<td>0.47</td>
</tr>
<tr>
<td>Compartment&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9.7 (7.9)</td>
<td>-2.2 (10.6)</td>
<td>11.4 (4.5)</td>
<td>-2.7 (6.0)</td>
<td>0.34</td>
</tr>
<tr>
<td>2</td>
<td>9.8 (6.5)</td>
<td>-3.0 (8.4)</td>
<td>10.9 (5.6)</td>
<td>-4.2 (6.8)</td>
<td>0.41</td>
</tr>
<tr>
<td>3</td>
<td>4.5 (3.6)</td>
<td>-0.8 (6.0)</td>
<td>4.1 (4.1)</td>
<td>-0.6 (5.3)</td>
<td>0.99</td>
</tr>
<tr>
<td>4</td>
<td>1.6 (2.2)</td>
<td>-0.5 (2.6)</td>
<td>1.5 (1.6)</td>
<td>0.0 (1.7)</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>0.3 (0.6)</td>
<td>-0.1 (0.5)</td>
<td>0.5 (0.9)</td>
<td>-0.3 (0.9)</td>
<td>0.62</td>
</tr>
<tr>
<td>$\Phi$&lt;sup&gt;f&lt;/sup&gt;</td>
<td>5.6 (6.4)</td>
<td>-0.7 (6.3)</td>
<td>5.6 (5.3)</td>
<td>-1.2 (4.3)</td>
<td>0.63</td>
</tr>
<tr>
<td>Height of highest labeled cell</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As cell No.</td>
<td>32.4 (8.6)</td>
<td>0.9 (12.8)</td>
<td>36.4 (13.5)</td>
<td>-4.6 (13.2)</td>
<td>0.37</td>
</tr>
<tr>
<td>As % of crypt height</td>
<td>52.5 (10.4)</td>
<td>-0.3 (11.8)</td>
<td>53.3 (13.5)</td>
<td>-5.9 (14.0)</td>
<td>0.89</td>
</tr>
</tbody>
</table>

* Two-month follow-up minus baseline differences.

<sup>a</sup> From analysis of covariance, with sex as a covariate for differences in baseline values and in follow-up minus baseline differences: placebo versus calcium.

<sup>b</sup> Proportion of cryp cells that are labeled; only S-phase cells were scored as labeled.

<sup>c</sup> Proportion of crypt cells that are labeled; only S-phase cells were scored as labeled.

<sup>d</sup> Crypt divided into fifths based on height of crypt by cell count, with compartment 1 being the lowest or basal compartment and compartment 5 being the highest or luminal compartment.

<sup>e</sup> Proportion of labeled cells that are in the upper 40% of the crypt; only S-phase cells were scored as labeled.

**Discussion**

This pilot study provides no substantial evidence that 2.0 g of calcium as calcium carbonate daily can normalize either the rate corresponding to proportional differences of −23% and −26%, respectively; thus, the LI in the calcium group was reduced, proportionately, 3% more than that in the placebo group ($P = 0.91$). For the $\Phi$, the absolute follow-up minus baseline differences for the placebo and calcium groups were −0.7% and −1.2%, respectively, corresponding to proportional differences of −12% and −22%, respectively; thus, the $\Phi$ in the calcium group was reduced, proportionately, 10% more than that in the placebo group ($P = 0.85$).
or distribution of proliferating cells in the colon crypts of patients with ulcerative colitis over a 2-month treatment period. However, it must be emphasized that the sample size was small and that findings may be due to chance. Other possible reasons for our findings include that (a) calcium may indeed have no substantial effect on colon cell proliferation in ulcerative colitis patients, because the colon carcinogenic processes in ulcerative colitis patients differ from those in sporadic adenoma patients in ways that involve or do not involve calcium, and (b) in contrast to our findings for adenoma patients, the calcium dose or duration may have been insufficient for efficacy. Given the suggestion of a 3% proportionally greater drop in the LI and the 10% proportionately greater drop in the $\Phi_h$, as well as the caveats about sample size and chance and calcium dose and duration, the possibility that calcium may beneficially contribute to a multifactorial approach to normalizing colorectal epithelial cell proliferation and reducing the risk of colon cancer in ulcerative colitis patients cannot be excluded.

If the true possible treatment effects were the sizes estimated by this pilot study, a sample size of 718 per treatment group would have been required to detect the difference in the LI at $P = 0.05$, and 368 per group would have been required to detect the difference in the $\Phi_h$. To detect perhaps more meaningful proportional differences of 20% in the LI and $\Phi_h$, the sample sizes would have needed to be 40 and 89 per group, respectively.

Other findings from this study include: (a) both the LI and $\Phi_h$ in ulcerative colitis patients are, on average, higher than those in sporadic adenoma patients; (b) investigating colorectal epithelial cell proliferation in ulcerative colitis patients in general, and in clinical trials and using PCNA in particular, is feasible; (c) the BrdUrd method for labeling proliferating colon crypt cells in ulcerative colitis is ineffective when used in the usual manner (the cause of this is unknown); and (d) the inadequate biopsy rate in the present study was 15% (compared to 5% in our sporadic adenoma patients; Ref. 45), a factor that should be considered in deciding on sample sizes for colorectal epithelial cell proliferation studies in ulcerative colitis patients.

The present study has several strengths and limitations. The most obvious limitation is, as pointed out above, the small sample size and the consequent increased role for chance in the likelihood of detecting (or not detecting) a treatment effect. The small sample size also precluded subgroup analyses. Another limitation, common to nearly all colorectal epithelial cell proliferation trials, is that the only site sampled was the rectum; thus, treatment effects higher in the colon cannot be ruled out. This is potentially important, because the physiology of the colon and rectum and the epidemiology of colon and rectal cancer differ (2). The strengths of the study are that it is, to our knowledge, the first clinical trial of calcium and colorectal epithelial cell proliferation in ulcerative colitis patients; the high level of protocol adherence; the strict quality control and consequent high level of biologic reliability; and, perhaps most importantly, the randomized, controlled design.

There have been no previous reports of trials of calcium and colorectal epithelial cell proliferation; however, as reviewed extensively elsewhere (49), there have been 14 previous such trials in patients without inflammatory bowel disease who were at increased risk for colon cancer (32, 39–47, 50–53). The results of these studies are inconsistent with one another: the majority of the controlled trials found mostly large decreases in the LI and $\Phi_h$, the small controlled trials yielded mixed results, and the full-scale controlled trials found small (not statistically significant) proportional decreases in the LI of about 3%. The full-scale trial in sporadic adenoma patients conducted by our group concurrently with the present study found a statistically significant proportional decrease of 100% in the $\Phi_h$ (45), but a second full-scale trial (47), a trial with more methodological problems (49), did not.

There have been no previous reports of comparisons between colorectal epithelial cell proliferation in ulcerative colitis patients and sporadic adenoma patients, but three small studies (12, 23, 24) compared ulcerative colitis patients with normal patients. All three studies used radiolabeled thymidine uptake and microautoradiography as the S-phase cell labeling method, reported the LI but not the $\Phi_h$, and did not report biopsy-scoring reliability. One study compared eight normal subjects to four patients with ulcerative colitis and found a 173% proportionately higher LI in the ulcerative colitis patients (9.5% versus 25.9%; SE and $P$ not given; Ref. 24). A second study compared four normal subjects with nine ulcerative colitis patients and found a 3% proportionately lower LI in the ulcerative colitis patients (11.1% versus 11.4%; SE and $P$ not given; Ref. 23). The third study compared eight normal subjects with seven ulcerative colitis patients and found a statistically significant 168% proportionately higher LI in the ulcerative colitis patients (9.5% versus 25.5%; SE 1.1 and 1.8, respectively; Ref. 12).

Taken altogether, the results of the present and past studies suggest that, on average, ulcerative colitis patients may have higher levels of colorectal epithelial cell proliferation than do normal or sporadic adenoma patients.

In summary, the sample size in this preliminary study does not allow a definitive answer to the question of whether calcium can reduce colorectal epithelial cell proliferation in ulcerative colitis patients; therefore, although the results suggest that calcium may have a modest effect, conservatively, they cannot be considered to support the hypothesis. This study suggests that ulcerative colitis patients have higher levels of colorectal epithelial cell proliferation than do sporadic adenoma patients.

### Table 3: Comparison of age-, sex-adjusted colorectal epithelial cell proliferation measurements in ulcerative colitis versus sporadic adenoma patients

<table>
<thead>
<tr>
<th>Biopsy measurement</th>
<th>Sporadic adenoma ($n = 119$)</th>
<th>Ulcerative colitis ($n = 28$)</th>
<th>Proportional difference* (%)</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-phase LI (%)</td>
<td>4.3 (0.3)</td>
<td>6.7 (0.6)</td>
<td>25.9%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>All-labeled cell LI (%)</td>
<td>16.1 (0.8)</td>
<td>31.1 (2.8)</td>
<td>93%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>S-phase $\Phi_h$ (%)</td>
<td>9.6 (0.6)</td>
<td>7.8 (1.5)</td>
<td>-3%</td>
<td>0.21</td>
</tr>
<tr>
<td>All-labeled cell $\Phi_h$ (%)</td>
<td>10.1 (0.8)</td>
<td>12.0 (1.9)</td>
<td>19%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*100% $\times$ (LI$\text{adenoma} - \text{LI}_{ulcerative \text{colitis}}$)/LI$\text{adenoma}$; similarly for $\Phi_h$.  
*From analysis of covariance; adjusted for age and sex.  
Proportion of crypt cells that are labeled, only S-phase cells were scored as labeled.  
Proportion of labeled cells that are in upper 40% of the crypt; only S-phase cells were scored as labeled.  
Proportion of labeled cells that are in the upper 40% of the crypt; all labeled cells were scored as labeled.
confirms the feasibility of colorectal epithelial cell proliferation trials in ulcerative colitis patients; and points out the futility of using BrdUrd labeling in ulcerative colitis patients, the feasibility of using PCNA labeling in these patients, the need to consider a biopsy inadequacy rate of approximately 15%, and the importance of randomization and control groups in chemoprevention trials of colorectal epithelial cell proliferation or other cancer-related end points.

Acknowledgments

We thank Colleen Forster for help with developmental work to adapt the PCNA labeling technique for large-scale studies; Patricia Winkels for advice and work on biopsy procurement and initial handling methods; Bryan Randall for development of the computer software necessary to score biopsy slides processed by the PCNA technique; and the physicians of Digestive Healthcare, PA for advice and work on biopsy procurement methods and implementing epidemiological studies at the university-private community interface that measure colorectal epithelial cell proliferation.

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Calcium and colorectal epithelial cell proliferation in ulcerative colitis.

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