Rectal Mucosal Proliferation, Dietary Factors, and the Risk of Colorectal Adenomas

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Abstract
Colorectal cancer arises from a series of precursor stages, the so-called adenoma-carcinoma sequence. Increased rectal mucosal proliferation may be an early step in this sequence. Because dietary factors are implicated in the etiology of colorectal cancer, one might predict that diet would also be associated with proliferation. We conducted this study to examine the association of diet with rectal mucosal proliferation. Rectal mucosal proliferation was measured in endoscopic biopsy specimens by proliferating cell nuclear antigen (PCNA) immunohistochemistry and whole crypt mitotic counts (WCMCs). Diet was evaluated using a validated quantitative food frequency questionnaire. The correlation between PCNA labeling index (LI) and WCMCs was determined using Kendall's $\tau$, a nonparametric measure of correlation. Logistic regression was used to examine the effect of proliferation on adenoma status, controlling for confounders. The relationship between proliferation and dietary and demographic factors was examined using linear regression. There were 308 patients who had one or both measures of proliferation. There was no significant correlation between PCNA LI and WCMCs (Kendall's $\tau = 0.04; P = 0.35$). Neither measure of proliferation was predictive of adenoma status, even after adjusting for potential confounders. Body mass index and calories per day were significant predictors of WCMCs ($P = 0.01$ and $P = 0.03$, respectively). PCNA labeling index was not associated with any dietary variables, although its association with dietary fat nearly reached statistical significance ($P = 0.09$). The association between proliferation and diet were generally inconsistent. There appears to be no simple relationship between colorectal cancer risk factors, colorectal adenomas, and these two measures of rectal mucosal proliferation. We need simpler, more reliable intermediate markers for use in etiological and intervention studies.

Introduction
Colorectal cancer is one of the leading causes of cancer death with about half a million deaths and more than 700,000 new cases diagnosed worldwide each year (1). It is generally believed that colorectal cancer arises from a series of recognizable pathological precursor stages, the so-called adenoma-carcinoma sequence. Mucosal hyperproliferation and an expansion of the proliferative zone in colonic crypts are thought to be an early step in the sequence (2). There are several lines of evidence that suggest that increased proliferation is related to carcinogenesis in the large bowel. Increased mucosal proliferation, for example, has been correlated with the risk of colon cancer in humans (3) and experimental animals treated with carcinogens (4). Populations at low risk of colorectal cancer such as the Seventh-day Adventist vegetarians have been shown to have lower proliferative index and low incidence of colorectal cancer (5). Based on these observations, cell proliferation has been suggested as a potentially useful intermediate or surrogate marker in studies of colorectal carcinogenesis (6, 7).

Several techniques have been used to measure rectal mucosal proliferation. Proliferation has traditionally been measured using the tritiated thymidine incorporation assay, but this method has the disadvantage of requiring the use of radioactive material and a long processing time. The results are not rapidly available. In comparison, immunohistochemical methods are less time-consuming and do not require the use of radioactive material. PCNA is a protein that is maximally synthesized during the S-phase of the cell cycle. PCNA immunohistochemistry can be used to measure proliferation by determining the proportion of labeled cells in the colonic crypt. The WCMC is another method used to evaluate mucosal proliferation in colon biopsies by counting the number of mitotic cells in dissected whole crypts. This method is relatively easy because it does not require extensive processing. Tissue orientation is not important, and a reasonable number of crypts can be easily counted from a single endoscopic biopsy (8).

There is considerable international variation in the incidence rates of colorectal cancer. Environmental factors, diet in particular, may account for this variation (9). Diets high in fat and low in fruits and vegetables have been linked with increased risk of colorectal cancer (10, 11). If increased rectal

$^1$ The abbreviations used are: PCNA, proliferating cell nuclear antigen; WCMC, whole crypt mitotic count; LI, labeling index; BMI, body mass index; OR, odds ratio; CI, confidence interval.
mucosal proliferation is an intermediate step in cancer development, then one might speculate that dietary factors that influence colorectal cancer risk might also be associated with proliferation. The present study was designed to examine the correlation between two different measures of proliferation, their ability to predict adenoma risk, and their correlation with reported risk factors for colorectal cancer.

Materials and Methods
Participants were drawn from patients who were referred to the University of North Carolina Hospitals for a clinically indicated colonoscopy between August 1992 and July 1995. Participants were enrolled in the study if they agreed to have biopsies taken from their large bowel during the procedure, have a blood sample drawn, participate in a telephone interview, and give informed consent. The study was approved by the Committee on the Protection of the Rights of Human Subjects at the University of North Carolina School of Medicine. Exclusion criteria included age <30 years, previous colon resection or cancer, polyposis, colitis, incomplete examination (i.e., cecum not reached), and unsatisfactory prep. Patients with a previous adenoma were included in the study. Cases were defined as individuals with one or more adenomatous polyps. Control subjects had no adenomatous polyps. The patients with previous adenoma but no current adenoma formed a separate comparison group.

Eligible subjects were interviewed about their diet over the telephone by a trained interviewer using the validated quantitative food frequency questionnaire developed at the National Cancer Institute (12). The interviewer was blind to the status of the subject as a case or control. Subjects were asked about their intake of over 100 foods. They estimated how often they consumed each food and their portion size for each item (small, medium, or large). Nutrient intake was calculated by an analysis program that incorporates the nutrient content of each food item, the consumption frequency, and a portion size based on age. The program was provided by the National Cancer Institute.

The biopsies for PCNA immunohistochemistry and WCMC were obtained and processed as described previously (13, 14). Briefly, six biopsies were taken from the rectum of eligible subjects at the start of the procedure using standard endoscopy forceps at ~8–12 cm from the anal verge. The biopsies were obtained in the morning or afternoon after an overnight fast. Four of the biopsies were carefully transferred to bilubus paper and placed in Steinberg’s modified Eagle’s medium for PCNA immunohistochemistry. Two specimens were placed in Carnoy’s fixative for cryt dissection (13). The specimens for PCNA immunohistochemistry were processed according to standard histological procedures. Five sections were placed on poly-L-lysine-coated slides (Sigma Chemical Co., St. Louis, Mo) and were taken at least 50 mm apart so that each would contain different crypts. Slides were incubated for 12–20 h at 4°C using PC10 antibody (DAKO Co., Carpenteria, CA). PCNA was detected using the BioGenex SterAvigen Super Sensitive kit for alkaline phosphatase (BioGenex, San Ramon, CA). The slides were counterstained with Mayer’s hematoxylin and mounted with Eukitt (Calibrated Instruments, Hawthorne, NY). Positive control slides in each batch of staining included rat testis and human colon tissue from a cancer patient.

The scoring criteria for PCNA has been described previously (13, 14). Crypts were selected for scoring if they were well oriented, if the entire length was visible in the longitudinal section, and if the base of the crypt touched the muscularis mucosa. If the muscularis was missing, a crypt was acceptable if its height was uniform with other crypts. The PCNA LI was calculated as a weighted average of the proportion of labeled cells in 16–24 crypts from two biopsies.

Tissue for whole crypt dissection was transferred from Carnoy’s fixative to 70% ethanol after 2.5–3.0 h. Prior to staining, the tissue was rehydrated in 50 and 25% ethanol and then hydrolyzed in 1 m HCl at 60°C for 10 min. The biopsies were then placed in Schiff’s reagent and incubated in the dark for 45–60 min at room temperature. A small piece of the biopsy was then placed on a microscope slide with 45% acetic acid and dissected under a dissecting microscope. The dissected crypts were examined under a compound microscope at ×200. To be scored, the top and bottom of the crypt had to be seen. Broken crypts were not acceptable. Mitotic cells were identified by nuclear clumping, focusing through the entire crypt. Each scorable crypt was traced on paper using a drawing attachment (Olympus Corporation, Lake Success, NY). The positions of all mitoses were noted within the crypt. For straight crypts, the height of each crypt was measured from the lowest point to the center of a line drawn through the apex. Curved crypts were measured using a flexible curve. The position of each mitosis was measured from the crypt base. WCMC was determined as the average of the number of mitotic cells per crypt in 20 crypts from two biopsies.

All data were entered and sight verified (i.e., a different person independently compared the data on the screen with the paper copy). Range and logic checks were performed to ensure the quality of the data. All statistical tests of significance were two-sided. The data analysis was centered on three principle aims: (a) to assess the degree of correlation between proliferation as measured by WCMC and proliferation as measured by PCNA LI; (b) to determine whether the proliferation indices, independently or in combination, predicted adenoma risk; and (c) to determine the relationships between proliferation and a variety of dietary and lifestyle factors.

The degree of correlation between the two proliferation measures was assessed using Kendall’s τ, a nonparametric measure of correlation (15). A nonparametric measure of correlation was selected because of the inherent differences in the two measures; PCNA LI is a rate, whereas WCMC is a count. Correlations were also computed separately by adenoma status. We used a variance components analysis to compute the proportions of variation between subjects, between biopsies within subjects, and within biopsies (16). For this analysis, the outcome for each subject was represented by an array of proliferation indices, arising from readings of multiple crypts within up to two biopsies, with case status included in the model as a fixed effect.

To determine how the proliferation indices relate to adenoma risk, we first examined the mean levels of proliferation by case status, separately for each measure of proliferation. Proliferation levels among the three groups (current adenomas, past adenomas, and never adenomas) were compared first in an unadjusted analysis using ANOVA techniques, separately for each measure of proliferation. We also examined mean levels of proliferation among those cases with at least one large adenoma (≥1.0 cm), case without a large adenoma, and controls. Analysis of covariance was used to compare the three groups (current adenomas, previous adenomas, and controls) with respect to proliferation, adjusting for age, race, and sex.

For PCNA, compartmental analysis was used to compare \( \phi_{ao} \) (17), the proportion of proliferating cells occurring in the upper (luminal) 40% of the crypt, among the three case/control
groups. Ordinal cell counts were used to identify which cells lay in the upper 40% of the crypt. ANOVA and analysis of covariance were used to compare the three groups first in an unadjusted analysis and then adjusting for age, race, and sex.

Logistic regression models were also used to examine the joint effects of PCNA LI and WCMC on adenoma risk. Both measures as well as their interaction were included as independent variables in a logistic model with case status as the outcome. Individuals with a history of adenoma were excluded from the logistic regression analyses; therefore, the response was either case (adenoma) or control (no adenoma history). Age, race, and sex were included as covariates in these models. To determine whether there might be a nonlinear relationship between proliferation and adenoma status, logistic regression models with adenoma risk modeled as a quadratic function of proliferation were also considered.

Our third goal was to examine the relationships between proliferation as measured by PCNA LI or WCMC and various dietary and lifestyle factors. For these analyses, proliferation was the outcome in linear regression models with possible predictors of proliferation considered as independent variables. Analyses were univariate, i.e., each of the proliferation measures was considered separately. Normalizing transformations were considered for both PCNA LI and WCMC. These analyses were performed for cases and controls separately in addition to the combined group. If the relationship between various lifestyle and dietary factors were different for cases than for controls, the results of these separate analyses would differ. Backward stepwise regression methods were used to determine which of the dietary and lifestyle factors were predictive of proliferation. Variables considered in the stepwise process were age (continuous), sex, race (white versus non-white), BMI (weight(kg)/height(m^2)), daily caloric intake, family history of colon cancer (yes/no), fat intake, fiber intake, alcohol drinker (yes/no), smoking (current, past, or never), laxative use (never, occasionally, once per month, once per week, or one or more times per day), constipation (yes/no), and number of bowel movements per week. Fat and fiber intake were adjusted for daily caloric intake using the method of Willett and Stampfer (18). All of the potentially predictive factors were included in a preliminary model. At each step, the least significant of these factors was eliminated from the model, and parameter estimates were recalculated until contrasts for all of the remaining factors had associated Ps of <0.10.

The proliferation measure taken as the outcome in the regression analyses just described is a weighted average of multiple measures of proliferation, and the unit of observation is a subject. As an alternative way of analyzing these data, mixed model analyses with the crypt as the unit of observation were carried out (16). These models account for the inherent within-subject correlation in proliferation. Terms for biopsy (within subject) and subject were included in these models. A compound symmetric model of the covariance structure was assumed; all observations were assumed to have the same variance, the covariance between any two measures taken from the same biopsy was assumed to be the same across subjects, and observations arising from different subjects were assumed to be uncorrelated. As with the linear regression analyses, a backward stepwise procedure was used to identify those variables that may be related proliferation. The results of the backward stepwise procedure obtained using the mixed model analyses were compared with those obtained with linear regression analysis. This was done for the case-only analysis with PCNA LI and then WCMC as the response.

### Table 1: Descriptive characteristics of study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall (n = 308)</th>
<th>Current adenomas (n = 114)</th>
<th>Previous adenoma (n = 57)</th>
<th>Controls (n = 137)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>59.9 (0.7)</td>
<td>62.4 (1.0)</td>
<td>63.0 (1.4)</td>
<td>55.7 (1.0)</td>
</tr>
<tr>
<td>White (%)</td>
<td>81.5</td>
<td>86.0</td>
<td>82.5</td>
<td>77.4</td>
</tr>
<tr>
<td>Male (%)</td>
<td>48.1</td>
<td>54.4</td>
<td>43.9</td>
<td>44.5</td>
</tr>
<tr>
<td>BMI [wt (kg)/ht(m^2)]</td>
<td>27.4 (0.3)</td>
<td>27.4 (0.5)</td>
<td>27.5 (0.7)</td>
<td>27.3 (0.4)</td>
</tr>
<tr>
<td>Family history (%)</td>
<td>19.2</td>
<td>21.1</td>
<td>21.1</td>
<td>16.8</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>81.2 (2.2)</td>
<td>80.6 (4.0)</td>
<td>72.9 (4.2)</td>
<td>85.2 (3.5)</td>
</tr>
<tr>
<td>Fiber (g/day)</td>
<td>14.4 (0.4)</td>
<td>14.3 (0.7)</td>
<td>14.6 (0.9)</td>
<td>14.5 (0.6)</td>
</tr>
<tr>
<td>Calories/day</td>
<td>1868 (40)</td>
<td>1847 (68)</td>
<td>1745 (77)</td>
<td>1937 (63)</td>
</tr>
<tr>
<td>Alcohol use (%)</td>
<td>47.4</td>
<td>47.4</td>
<td>54.4</td>
<td>44.5</td>
</tr>
<tr>
<td>Smokers (%a)</td>
<td>22.3</td>
<td>19.5</td>
<td>19.3</td>
<td>25.9</td>
</tr>
<tr>
<td>Bowel movements/week</td>
<td>9.6 (0.5)</td>
<td>9.5 (0.5)</td>
<td>11.6 (1.8)</td>
<td>8.9 (0.6)</td>
</tr>
<tr>
<td>Laxative use (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>57.9</td>
<td>59.5</td>
<td>64.9</td>
<td>53.7</td>
</tr>
<tr>
<td>Occasionally</td>
<td>19.9</td>
<td>21.6</td>
<td>12.3</td>
<td>21.6</td>
</tr>
<tr>
<td>Once per month</td>
<td>7.9</td>
<td>7.2</td>
<td>5.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Once per week</td>
<td>5.6</td>
<td>5.4</td>
<td>5.3</td>
<td>6.0</td>
</tr>
<tr>
<td>Ever constipated (%)</td>
<td>8.6</td>
<td>6.3</td>
<td>12.3</td>
<td>9.0</td>
</tr>
<tr>
<td>Ever constipated (%)</td>
<td>58.8</td>
<td>56.6</td>
<td>63.2</td>
<td>58.8</td>
</tr>
</tbody>
</table>

*One person in the current adenoma group and two controls did not answer the question about smoking.

*Three people in the current adenoma group and three controls did not answer the question on laxative use.

*One person in the current adenoma group and one control did not answer the question about constipation.

### Results
There were 3488 colonoscopies performed between August 15, 1992 and July 31, 1995; 1572 patients were ineligible and therefore not asked to participate. An additional 115 gave consent but were found to be ineligible at the time of colonoscopy (mostly commonly due to poor preparation). There were 707 subjects who were eligible but were not asked to participate (mostly because the nursing staff were too busy with primary patient care responsibilities) and 602 who were eligible but refused. A total of 492 subjects met eligibility criteria and patient care responsibilities) and 602 who were eligible but refused. A total of 492 subjects were eligible but were not asked to participate (mostly because the nursing staff were too busy with primary patient care responsibilities) and 602 who were eligible but refused. A total of 492 subjects met eligibility criteria and were enrolled in the study. Of these 492 subjects, there were 393 subjects with proliferation information. For those 99 subjects missing the proliferation information, biopsies were either inadequate or no specimen was collected. After deleting data from people found to have cancer, 381 subjects remained. Of these, 308 completed the diet questionnaire, had dietary data judged by the interviewer to be of good quality, and thus were included in our analyses. The descriptive characteristics of the study subjects, overall and by case status, are shown in Table 1.

We first examined the correlation between the two measures of proliferation. There was no significant correlation between PCNA LI and WCMC (Kendall’s τ = 0.04; P = 0.35). Among current adenoma cases, the correlation between PCNA LI and WCMC was 0.12 (P = 0.10). Among individuals with a history of adenomas, the correlation between PCNA LI and WCMC was also 0.12 (P = 0.28). Among controls with no history of adenomas, there was a slight negative correlation, −0.07 (P = 0.30). Graphs revealed little relationship between the two measures, either overall or for any of the case/control comparisons, in agreement with the correlation statistics (Fig. 1).

Between-subject variation accounted for 61% of the variation in WCMC compared with 39% for PCNA LI. Variation between biopsies within subjects accounted for 9% of the
variance in WCMC compared with 21% for PCNA LI. The
remaining variation (30% for crypt dissection and 40% for
PCNA LI) occurred between crypts and within biopsies.

Examination of the distributions of WCMC and PCNA LI
led to the selection of a natural log transformation for normal-
izing WCMC measures and a square root transformation for
normalizing PCNA LI measures. These transformations were
used for parametric analyses requiring normality. ANOVA
revealed no significant difference in proliferation among the
three groups, current adenoma (CA), previous adenoma (PA),
and never adenoma (NA) for either PCNA LI or WCMC. The
overall unadjusted mean and SE for PCNA LI was 7.2% (0.25%),
WCMC was 5.58% (0.33%), and \( \phi_{ao} \) was 3.8% (0.25%). Means and SEs for each proliferation measure by case
status are shown in Table 2. Analysis of covariance, adjusted
for age, race, and sex, also did not reveal any difference
among the three groups. Least square means and SEs, adjusted
for age, race, and sex, were determined using a model without transformation of the variables and are shown in Table 2. \( P \) for comparison resulted from the model using transformations. Analysis of \( \phi_{ao} \), the proportion of proliferating cells which occur in the luminal compartment of the crypt, revealed no difference among the three groups, either in unadjusted or adjusted analysis (Table 2).

Further comparisons of PCNA LI, WCMC, and \( \phi_{ao} \) be-
tween cases with at least one large adenoma (i.e., 1.0 cm in
diameter or larger), cases without a large adenoma, and controls
revealed no differences among the three groups. There were,
however, small numbers of cases with large adenomas; 14 for
the PCNA LI and \( \phi_{ao} \) comparison and 13 for WCMC.

Logistic models were also used with case status as the
outcome (current adenomas versus the never adenoma group
only) to examine the effect of proliferation on case status and
in particular to allow for a quadratic relationship between
proliferation and adenoma status. Variables for age, sex, and
race were included in these models. The quadratic terms con-
sidered were not significant when either PCNA LI or WCMC
were considered separately. \( Ps \) for the quadratic term were 0.94
and 0.26 for PCNA LI and WCMC, respectively.) For the
model with a linear term for PCNA LI, the OR comparing the
75th percentile of the distribution of PCNA LI to the 25th
percentile was 0.83 (95% CI, 0.58–1.20). This implies that a
high PCNA LI is slightly protective, although not significantly
so. The model with a linear term for WCMC returned an OR of
1.08 (95% CI, 0.75–1.57) when comparing an observation at the
75th percentile of the distribution to one at the 25th percent-
ile. When both PCNA LI and WCMC, as well as their interaction,
were included in a logistic model, their interaction term was nearly significant (\( P = 0.09 \)). To examine the com-
bined effect of WCMC and LI at high and low levels of each,
we computed the OR comparing cases to the never adenoma
group for various combinations of low (defined at the 25th
percentile) and high (defined as the 75th percentile) values of
WCMC and LI. The 25th percentile of both WCMC and LI was
taken as the reference. The resulting ORs are shown in Table 3.
Those at the 75th percentile for both WCMC and LI were less
likely to have an adenoma than those at the 25th percentile for
both WCMC and LI (OR, 0.84; 95% CI, 0.48–1.49).

We examined the relationship between proliferation and
dietary and lifestyle variables separately for the combined
group (CA, PA, and NA) using linear regression with transfor-
mations done to produce normality. The initial model included
covariates. The results are shown in Table 4. In these full
models, BMI and calories were significant when WCMC was
the response. With PCNA LI as response, there were no sig-
ificant associations. We next performed backwards stepwise
regression. The results of this backward stepwise process are
listed in Table 5. For WCMC, calories and BMI remained in the
final model, and both were positively associated with WCMC.
For PCNA LI, only fat remained at the end of the stepwise
procedure, and its association with PCNA LI was in a positive
direction. The results of mixed model analysis with the crypt as
the unit of observation were in agreement with those obtained
using linear regression for the cases-only analysis with WCMC
and then with PCNA LI as the response. Because we obtained
similar results with mixed model analysis for this group of
subjects, we used only the simpler method of linear regression
with the subject as the unit of observation for further analyses.

For the case-only analysis, only BMI remained in the
model with WCMC as the response. Its association with
WCMC was in a positive direction. For PCNA LI, daily fiber
intake and number of bowel movements per week remained
after the backward stepwise procedure. Increased fiber was
associated with lower PCNA LI and number of bowel move-
ments per week was positively associated with PCNA LI. For
analyses including only controls, male gender was positively
associated with WCMC and fat was positively associated with
PCNA LI. All other factors were excluded during the backward
stepwise process.

**Discussion**

In the present study, we found a poor correlation between two
commonly used proliferation markers, no association between
proliferation index and the presence of adenomas, and incon-
sistent associations between proliferation indices and environ-
mental factors (dietary/other lifestyle) that have been linked
with risk for colon cancer. Although there is presently consid-
erable interest in the use of surrogate end point biomarkers as
measures of colon cancer risk (19), our results suggest that
enthusiasm for the use of these markers in etiological or che-
moprevention studies must be tempered. Measuring rectal mu-

![Fig. 1. Scatter plot of labeling index against WCMC, all subjects. \( r = 0.04; P = 0.35 \).](image-url)
Table 2 Unadjusted and adjusted means (SE) of proliferation measures

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Current adenoma</th>
<th>Previous adenoma</th>
<th>Controls</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>PCNA LI</td>
<td>6.7% (0.38%)</td>
<td>8.1% (0.67%)</td>
<td>7.3% (0.38%)</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>WCMC</td>
<td>5.82% (0.33%)</td>
<td>5.16% (0.36%)</td>
<td>5.55% (0.23%)</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>( \phi_0 )</td>
<td>3.9% (0.38%)</td>
<td>3.3% (0.42%)</td>
<td>3.8% (0.35%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Adjusted ( a )</td>
<td>PCNA LI</td>
<td>7.1% (0.48%)</td>
<td>8.5% (0.65%)</td>
<td>7.5% (0.42%)</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>WCMC</td>
<td>6.00% (0.32%)</td>
<td>5.43% (0.45%)</td>
<td>5.61% (0.29%)</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>( \phi_0 )</td>
<td>4.0% (0.45%)</td>
<td>3.4% (0.57%)</td>
<td>4.1% (0.38%)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

* For PCNA LI and \( \phi_0 \), \( n = 284, 104, 51, \) and 129 for the overall, current adenoma, previous adenoma, and control groups, respectively.

* Obtained using ANOVA for unadjusted analyses and ANCOVA for adjusted analyses. Normalizing transformations were used for PCNA LI and WCMC.

\( a \) Adjusted for age, race and sex.

Table 3 ORs of current adenoma versus never adenoma for various levels of PCNA LI and WCMC, while controlling for the PCNA LI-WCMC interaction as well as age, sex, and race. The reference is the 25th percentile of both PCNA LI and WCMC.

<table>
<thead>
<tr>
<th>Level of PCNA LI</th>
<th>Level of WCMC</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>25th percentile = 0.040</td>
<td>25th percentile = 3.65</td>
<td>1.0</td>
<td>0.44-2.16</td>
</tr>
<tr>
<td>25th percentile = 0.040</td>
<td>75th percentile = 6.95</td>
<td>0.75</td>
<td>0.43-1.34</td>
</tr>
<tr>
<td>75th percentile = 0.089</td>
<td>25th percentile = 3.65</td>
<td>0.66</td>
<td>0.39-1.13</td>
</tr>
<tr>
<td>75th percentile = 0.089</td>
<td>75th percentile = 6.95</td>
<td>0.84</td>
<td>0.48-1.49</td>
</tr>
</tbody>
</table>

Another possible explanation for the lack of correlation between the two markers may be the increase in age, race and sex. The correlation between the two markers may be explained, in part, by the fact that they measure different components of the cell cycle. WCMC measures a narrow segment (mitosis) of the cell cycle, whereas PCNA LI measures the G1 and S phases of the cell cycle (20). The disparate results with the two proliferation markers underscore the importance of recognizing that different markers have different performance characteristics. The results that are observed using one marker may not be seen with another marker. In the present study, we found isolated correlations between these measures of correlation and certain environmental exposures. The fact that we found different exposures for the different proliferation measures could either be due to chance or could mean that different environmental factors act on different parts of the cell cycle.

Another possible explanation for the lack of correlation between the two markers is measurement error. It is technically difficult to calculate these indices, and random errors in measurement would reduce the correlation. Proliferation assays are particularly labor intensive because of the need to locate and count many cells. The PCNA immunohistochemical assay used in this study requires proper orientation of the specimen to permit adequate visualization of the entire crypt column. WCMC, on the other hand, does not require orientation, and more crypts can be evaluated per biopsy with the WCMC assay. Although measurement errors could reduce the correlation between measures, we used very strict rules that defined scorable crypts and prescribed rules for counting. The same individual (T. O. K.) scored all of the crypts. Previous quality control work using samples from this study to assess the scoring reliability of these two measures of proliferation by our group showed an extremely high intrarater reliability (99%; Ref. 14). We do not believe that measurement error accounts for the lack of correlation.

We did not find any association between either measure of proliferation and the presence of adenomas. Previous investigators have reported that individuals with polyps and cancer have increased mucosal proliferation throughout the colon, a so-called "field effect" (21), and this abnormal proliferation in the colonic mucosa has been suggested to predispose to colonic neoplasia (22). Weisgerber et al. (23) reported a positive correlation between PCNA LI, patient age, and size of adenoma. Another study found a positive association between PCNA LI and recurrent adenomas (24). Methodological differences such as differences in laboratory protocols, scoring protocols, different staining intensities in the PCNA assay, PCNA antibody clone, concentration of antibody, and brand of immunohistochemical reagents may account for the discrepancy between our results and these studies. These results have not been confirmed by all investigators. Anti et al. (25), for example, found that LI as measured by tritiated thymidine assay had a low accuracy in distinguishing between polyp patients and controls. Rozen et al. also did not observe higher levels of proliferation among individuals with adenomas (26).

The proliferative zone in colonic crypts is normally confined to the basal two-thirds. A shift in the zone of proliferation...
Studies in animals appear to be much more successful in demonstrating differences in labeling indices. This may be due to the genetic similarity of the animals (they are often litter mates), the standard diets, and most importantly the fact that the animals are generally given carcinogens that substantially elevate the proliferation index above baseline. Our inability to detect associations in this study may be explained in part by the fact that in relatively healthy low-risk individuals, the within-subject variation in proliferation may be as large or larger than the between-subject variation. This makes it difficult to detect a difference between two groups of subjects. Other possible reasons for the lack of association between cell proliferation and potential risk factors for colorectal cancer could be that epithelial proliferation may not be a valid intermediate end point for colorectal cancer, and the risk factors for epithelial proliferation may vary from those steps further along in the carcinogenesis process (24). Epithelial proliferation in the colonic mucosa could perhaps still play some role as an intermediate biomarker of risk in colorectal cancer intervention trials (39, 40). We simply need more markers that are more reliable and more responsive than the ones presently available.

In summary, we found that PCNA LI and WCMC did not correlate with each other and were not predictive of the adenoma status. The associations between proliferation and diet were generally not consistent except that BMI and total calories were associated with WCMC. It appears that there is no simple relationship between colorectal cancer risk factors and these two measures of proliferation. We need simpler, more reliable intermediate markers for use in etiological and intervention studies.

### References


### Table 5 Factors remaining in model after backwards stepwise procedure with proliferation as response

<table>
<thead>
<tr>
<th>Group</th>
<th>Outcome measure</th>
<th>Factor</th>
<th>P</th>
<th>Correlation coefficient</th>
<th>$R^2$ statistic (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All subjects</td>
<td>WCMC</td>
<td>BMI</td>
<td>0.01</td>
<td>0.23</td>
<td>4.42</td>
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<tr>
<td></td>
<td>WCMC</td>
<td>Calories</td>
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<td>0.20</td>
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</tr>
<tr>
<td>Cases only</td>
<td>WCMC</td>
<td>BMI</td>
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<td>7.81</td>
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<tr>
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<td>WCMC</td>
<td>PCNA LI</td>
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<td>7.81</td>
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<tr>
<td></td>
<td>WCMC</td>
<td>Fiber</td>
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<td>0.009</td>
<td>3.95</td>
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<tr>
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<td>Bowel</td>
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<td>0.77</td>
<td></td>
</tr>
<tr>
<td>Controls only</td>
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<td>Male gender</td>
<td>0.03</td>
<td>0.20</td>
<td>4.08</td>
</tr>
<tr>
<td></td>
<td>WCMC</td>
<td>Fat</td>
<td>0.09</td>
<td>0.03</td>
<td>0.10</td>
</tr>
</tbody>
</table>

* Partial correlation coefficient for models, which include more than one factor. Pearson correlation coefficient for models including only one factor.

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Rectal mucosal proliferation, dietary factors, and the risk of colorectal adenomas.


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