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Clinical Investigation

Modulation of Abnormal Colonic Epithelial Cell Proliferation and Differentiation by Low-Fat Dairy Foods

A Randomized Controlled Trial

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Context.—Before the development of human colonic neoplasms, colonic epithelial cells showed altered growth and differentiation. These alterations characterized mucosa at risk for cancer formation and were termed *intermediate biomarkers of risk*. Modifications of the mucosa toward more normal features by nutrients or drugs are putative approaches to chemoprevention of colon cancer.

Objective.—To determine whether increasing calcium intake via dairy products alters colonic biomarkers toward normal.

Design.—Randomized, single-blind, controlled study.

Setting.—Outpatient clinic.

Participants.—Seventy subjects with a history of polypectomy for colonic adenomatous polyps.

Intervention.—Low-fat dairy products containing up to 1200 mg/d of calcium. Subjects were randomized to 4 strata by diet (control vs higher calcium) and age (<60 vs ≥ 60 years).

Main Outcome Measures.—Changes in total colonic epithelial cells and number and position of thymidine-labeled epithelial cells and changes in the ratio of sulfomucins (predominantly secreted by distal colorectal epithelial cells) to sialomucins and expression of cytokeratin AE1, 2 markers of colonic cell differentiation.

Results.—During 6 and 12 months of treatment, reduction of colonic epithelial cell proliferative activity ($P<.05$), reduction in size of the proliferative compartment ($P<.05$), and restoration of acidic mucin ($P<.02$), cytokeratin AE1 distribution ($P<.05$), and nuclear size ($P<.05$) toward that of normal cells occurred. Control subjects showed no differences from baseline proliferative values at 6 and 12 months ($P>.05$).

Conclusion.—Increasing the daily intake of calcium by up to 1200 mg via low-fat dairy food in subjects at risk for colonic neoplasia reduces proliferative activity of colonic epithelial cells and restores markers of normal cellular differentiation.

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SINCE COLON CANCER claims 134 000 victims and causes about 55 000 deaths annually in the United States,[1] studies of potential chemopreventive nutritional regimens are an important objective of cancer research. Epidemiologic evidence suggests that dietary levels of calcium and vitamin D intake are inversely related to the incidence of colon cancer.[2-4] Supplementary dietary calcium inhibits colonic epithelial cell proliferation[5] and cytotoxicity of fecal water in rodent models and in humans[6-8] and colonic tumor formation in rodents.[9] Changes in cellular proliferation and differentiation are currently used as biomarkers to evaluate the effects of chemopreventive agents in human studies of colon cancer prevention.[5]

For editorial comment see p 1095.

In this report, subjects with previous colonic adenomas who were at greater risk for the development of colonic neoplasms than the general population[10] were studied to evaluate a dietary regimen of low-fat dairy foods resulting in high calcium intake. Subjects were randomized into 2 groups, an experimental group receiving dairy food supplementation and a control group maintaining their conventional diets. Subjects were then monitored for 1 year to evaluate changes in epithelial cells of the rectosigmoid mucosa describing the proliferative status, differentiation, and maturation of the cells.

METHODS

Subjects

Subjects older than 18 years with a history of colonic adenomatous polyps were studied. Exclusion criteria included a history of colon cancer, intestinal surgery other than appendectomy,

malabsorption, inflammatory bowel disease, significant endocrine or metabolic disorders, known lactose intolerance, a total calcium intake of more than 1000 mg/d, supplemental calcium of more than 400 mg/d, or supplemental vitamin D intake. Subjects were randomized to treatment by the method of randomly permuted blocks.[11] For each of the 4 subject strata defined by sex and age (≥60 vs <60 years), a separate treatment allocation scheme was created. In each scheme, equal numbers of patients were randomized to each treatment arm within blocks of size 2, 4, or 6 treatment assignments. The order of the blocks was randomly ordered within each scheme. Randomization sequences were produced using SAS Proc Plan (SAS Institute, Cary, NC).

Three hundred fifty potential volunteers with a recent history of adenomatous polyps were chosen from hospital records. The subject's primary care physicians permitted us to contact about 230 subjects as potential volunteers who were generally in good health and compliant. Of the 180 subjects who were actually contacted, about 65 had dietary exclusions, calcium supplementation, or a history of lactose intolerance; about 25 had medical reasons that barred their participation; and 20 were unwilling to participate. The 70 subjects that entered the study represented a cross-section of the initial potential volunteers. Of the 70 subjects, 33 were controls and 37 received dairy calcium supplements (Figure 1). These subjects were reimbursed for the inconvenience and expenses of coming to the hospital for the initial physical examinations, 4 nutrition interviews, and 4 visits for biopsies. The study was single-blinded since subjects knew they were on a special diet; however, they were unaware of the significance of their diets in relationship to the data sought. Estimate of sample size for the present study was based on our previous study of reduction of proliferative labeling index following calcium administration.[12] It was determined that in a randomized study an experimental group would require a sample size of 25 to 30 subjects in each arm to detect a significant difference from a control group maintaining a type I error at 5% with 80% power to detect a significant difference, using a 2-tailed test. The study was started in January 1993 and completed in June 1995.

Nutritional Assessment and Monitoring

Nutritional intake was assessed by a trained nutritionist using 24-hour food recalls and 3-day food diaries twice at baseline 1 month apart and then at 6-month intervals. Participants specifically included detailed information on dairy products. Food recalls also were performed at 3-month intervals, and discrepancies were investigated. Because of continual changes in product composition and food fortification, submitting food labels and brand names was required.

The experimental group of subjects were counseled individually with the target to increase daily dairy calcium content by 1200 mg, maintaining intake of fat, calories, and fiber constant by a suggested daily reduction of 45 g of carbohydrate, 30 g of protein, and 5 g of fat. Treatment participants were provided a nonfat processed cheese (Lifetime Fat-free Cheese, Lifeline Food Co, Seaside, Calif) and asked to increase intakes of other low-fat dairy products; no calcium supplements were permitted. Control subjects were counseled individually at 3-month intervals to maintain their pre-study diet.

Three-day diet records were analyzed by multirecord calculations using the Minnesota Nutrition Data System software, version 2.7 (Nutrition Coordinating Center, University of Minnesota, Minneapolis).

Rectosigmoid Biopsies

Rectosigmoid biopsy specimens were taken from "flat" endoscopically normal-appearing mucosa twice, 1 month apart, at baseline, and at 6 and 12 months. Fibersigmoidoscopy was performed between 8 and 10 AM after a tap water enema and 6 to 8 biopsy specimens were taken with jumbo forceps at 4 quadrants approximately 10 to 15 cm from the anal verge. Biopsy specimens were incubated with 0.185 MBq [^{3}H]thymidine[12] and fixed in 10% buffered formalin for studies of [^{3}H]thymidine incorporation into proliferating cells or 95% ethanol for cell differentiation markers.

Cell Proliferation and Differentiation Studies

Cell Proliferation.—Cell proliferation was studied on 15 to 25 well-oriented crypts as described in detail previously.[12] Total numbers of epithelial cells and numbers and positions of thymidine-labeled epithelial cells in each crypt column were recorded and then automatically processed with a computerized system.[13]

Studies of Differentiation Markers.—Acidic mucins were stained with a high-iron diamine solution[14] in 10 to 12 well-oriented crypts. Since sulphomucin is predominantly secreted by distal colorectal epithelial cells, abnormalities in mucins can be demonstrated by the proportion of sulphomucins to sialomucins.[14]

Cytokeratin AE1 expression was studied immunohistochemically using a monoclonal antibody to cytokeratin AE1.[14] Cytokeratin AE1 expression in epithelial cells was measured in 10 to 12 well-oriented crypts. In normal colorectal mucosa, cytokeratin AE1 expression is confined to the mucosal surface and the upper part of the crypt resulting in a low score for cytokeratin AE1 expression. Cytokeratin AE1 expression increases when the antigen expands to the lower crypt.

Nuclear Morphometric Measurements.—The dimensions of colorectal epithelial cells were measured in hematoxylin and eosin–stained, 3-μm sections

Table 1.—Study Subject Characteristics*

Characteristic	Control	Experimental
No. of subjects	33	37
Age, mean (SE), y	66 (2)	67 (3)
≥65	19	25
<65	14	12
Sex		
Male	22	22
Female	11	15
Race		
Black	8	12
White	19	21
Hispanic	5	4
Other	1	0
Weight, mean (SE), kg	76.5 (2.7)	79.4 (3.6)
Adenomas		
Time since last polypectomy, mean (SE), mo	7.2 (3)	8.6 (2)
Multiple polypectomy, %	14	16

*There were no significant differences in any determination between the total groups or the subjects that remained in the study at 1 year.

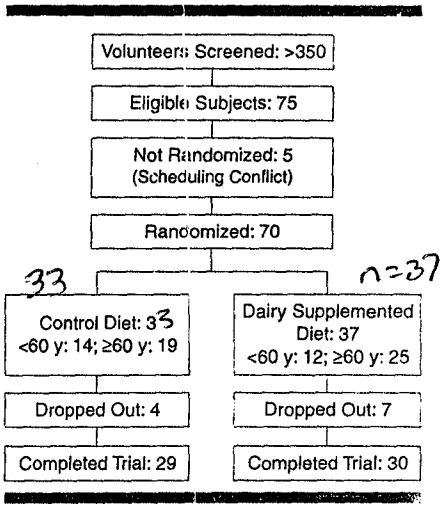

Figure 1.—Randomized study of colonic biomarkers from the screening process to completion. Of the 4 subjects who dropped out of the control diet group, 2 did so for personal reasons and 2 for medical reasons. Of the 7 subjects that dropped out of the dairy supplemented diet group, 2 did so for personal reasons, 1 for medical reasons, 2 for noncompliance, and 2 for intolerance to increased dairy intake.

Table 2.—Calculated Daily Intake of Selected Nutrients*

	Control†	Dairy‡	*P* Value
Energy, kJ			
Baseline	6745 (77)	7459 (84)	>.10
6 mo	6686 (74)	7001 (78)	>.10
12 mo	7279 (96)	7371 (73)	>.10
Protein, g			
Baseline	70.2 (4.3)	74.9 (3.3)	>.10
6 mo	70.2 (3.5)	82.1 (3.5)	<.02
12 mo	74.7 (4.7)	86.0 (4.0)	.07
Fat, g			
Baseline	49.9 (3.1)	60.0 (4.1)	.06
6 mo	49.5 (3.5)	45.7 (3.8)	>.10
12 mo	56.0 (6.3)	45.2 (3.2)	>.10
Calcium, mg			
Baseline	642 (48)	608 (29)	>.10
6 mo	725 (60)	1421 (76)	<.001
12 mo	685 (31)	1538 (103)	<.001
Phosphates, mg			
Baseline	1082 (59)	1073 (47)	>.10
6 mo	1106 (63)	1767 (100)	<.001
12 mo	1192 (104)	1915 (131)	<.001
Vitamin D, µg			
Baseline	5.2 (0.9)	4.4 (0.5)	>.10
6 mo	4.9 (0.6)	5.4 (0.4)	>.10
12 mo	4.7 (0.6)	5.8 (0.6)	>.10
Fiber, g			
Total			
Baseline	16.8 (1.7)	16.4 (1.0)	>.10
6 mo	17.2 (1.4)	16.0 (1.2)	>.10
12 mo	20.5 (2.4)	17.1 (1.1)	>.10
Insoluble			
Baseline	10.8 (1.2)	10.3 (0.7)	>.10
6 mo	11.0 (1.0)	10.1 (0.8)	>.10
12 mo	13.4 (1.8)	10.9 (0.8)	>.10

*Overall group mean (SE) daily intake of nutrients in the 2 study groups calculated from 3-day food diaries as described in "Methods." Numbers shown in parentheses denote the subjects evaluable at each time point.
†Number of control subjects for baseline values was 33; 6 months, 29; and 12 months, 29.
‡Number of dairy subjects for baseline values was 37; 6 months, 29; and 12 months, 30.

Table 3.—Data on Epithelial Cell Proliferation*

	Control, Mean (SE)			Treatment, Mean (SE)		
Variable	Baseline	6 mo	12 mo	Baseline	6 mo	12 mo
Total LI, %	5.5 (0.4)	7.1 (0.7)	6.6 (0.6)	6.0 (0.3)	6.3 (0.4)	5.1 (0.2)†
LI 4, %	4.0 (0.6)	3.4 (0.7)	3.5 (0.9)	3.5 (0.6)	3.6 (0.8)	1.9 (0.4)†
LI 5, %	0.6 (0.1)	1.0 (0.4)	0.5 (0.2)	0.8 (0.2)	0.3 (0.1)†	0.2 (0.1)†
Labeled cells, No.	2.8 (0.2)	3.6 (0.3)	3.6 (0.4)	2.9 (0.1)	3.1 (0.2)†	2.6 (0.2)†
Phi h, $\times 10^{-1}$	1.6 (0.2)	1.5 (0.4)	1.2 (0.2)	1.5 (0.2)	1.2 (0.2)	0.9 (0.2)

*LI indicates labeling index; Phi h, measure of distribution of the proliferative cells in the crypt. Significant group by time interaction occurred in total LI to 1 year, $P<.02$; LI 5% to 6 months, $P<.02$; in labeled cells to 1 year, $P<.01$; and Phi h to 1 year, $P<.01$.
†When compared with control, $P<.05$.

using a computer-assisted cell image analyzer (Samba 4000, Imaging Products International Inc, Chantilly, Va). Nuclear areas, perimeters, and diameters were evaluated in an average of 10 well-oriented crypts.

Statistical Analysis.—Twenty-four–hour nutrient consumption between study groups was compared at each time point (baseline, 6 months, and 12 months). The 2 baseline values obtained 1 month apart are averaged for each subject. Results between the study groups at each time point were compared by Student *t* tests. In addition, data for each nutrient and biomarker measure at the 3 time points for the 2 groups were analyzed by means of a 2-way, mixed-measures analysis of variance to determine the effect of dietary change or biomarkers over time. Time served as the repeated measure and the diet or biomarker served as the "between-group" factor. Post hoc Duncan multiple range tests were then performed on all significant main effects and interactions to test differences between group means at any time point ($P<.05$). To control for the possibility of altered fat consumption in the dairy-treated group, an additional analysis of covariance was performed on the biomarkers, which changed significantly over time, with total daily fat intake (grams) as the covariant. Adjusted least squares means were then compared between the groups over time.

RESULTS

Of the total of 70 subjects, 37 were entered into the experimental group and 33 were controls. The 2 groups were matched for demographics and time from prior polypectomy (Table 1). Eleven subjects dropped out of the study (7 were in the experimental and 4 in the control group). Dropouts occurred for medical or personal reasons without any difference between the 2 experimental groups (Figure 1).

The nutrient intakes in control and experimental subjects were similar at baseline (Table 2). Control subjects maintained their baseline nutrient intake including calcium throughout the study. Experimental subjects increased daily total calcium intake by a mean of 730 to 810 mg. Analysis of calcium from foods at baseline showed that 53% to 60% came from dairy sources (about 35% from milk, 6% from yogurt, and 16% from cheeses). Experimental subjects increased dairy sources of food calcium to 83% of intake (31% from milk, 11% from yogurt and ice cream, and 41% from cheese products). Energy intake was 1% to 5% greater in the supplemented subjects.

Evaluation of the 2 baseline rectal biopsy specimens permitted calculation of the reproducibility of biomarker measurements, which was used to help determine statistically significant changes during the study. There was no difference in proliferation kinetics except for the labeling index in 1 subcompartment of the crypt (compartment 3) ($P=.05$). The coefficients of variation of measurements between the 2 baseline biopsy specimens were 12.4% for cytokeratin AE1, 10% for acidic mucins, and 10.8% for total nuclear area morphometry. Duplicate intraobserver measurements showed these values: cytokeratin AE1, $r^2=0.969$; acidic mucins, $r^2=0.945$; nuclear morphometry, $r^2=0.865$; and proliferative indices, $r^2=0.966$.

At baseline, the 2 groups showed no significant differences in any proliferative parameter studied. At 6 and 12 months, control subjects also showed no differences from baseline proliferative values, and a trend was not detected. In contrast, significant changes were found in the study subjects. At 6 months, there were decreased numbers of proliferating cells throughout the whole crypt and a smaller proliferative compartment as shown by the decreased fraction of [^{3}H]thymidine-labeled cells in the upper 40% of the crypt adjacent to the luminal surface (phi h) and lower labeling index

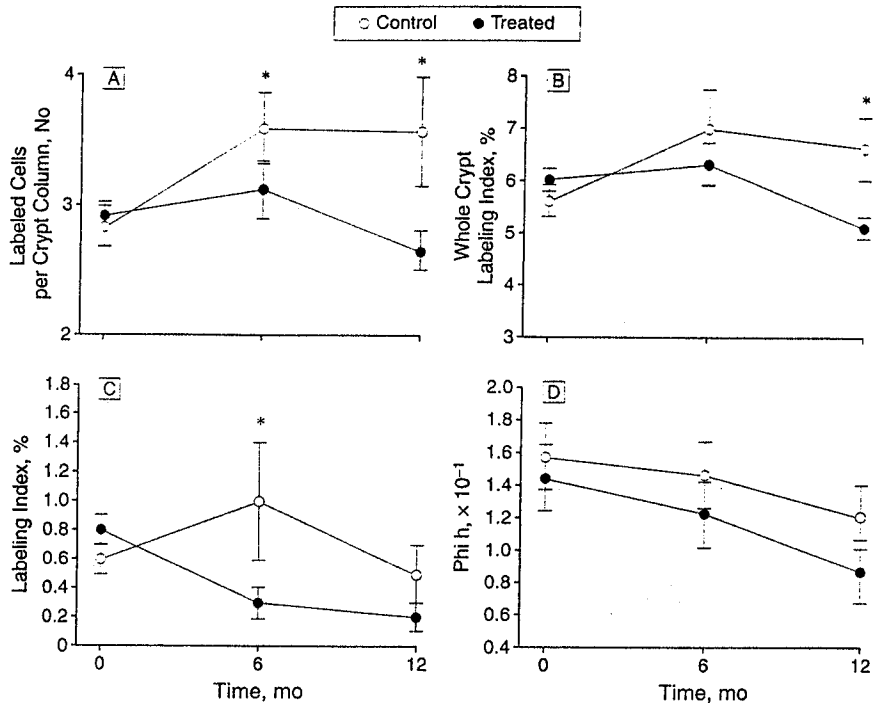

Figure 2.—Effect of dairy supplementation on cell proliferation determined in colorectal mucosa. Data shown as mean (SE) in the number of subjects studied at each time point. Asterisk indicates $P<.05$ for time between the groups. Part C is the labeling index of compartment 5 adjacent to the lumenal surface of the colonic crypts. Part D indicates Phi h, the fraction of proliferating cells in the upper 40% of the colonic crypts adjacent to the lumenal surface.

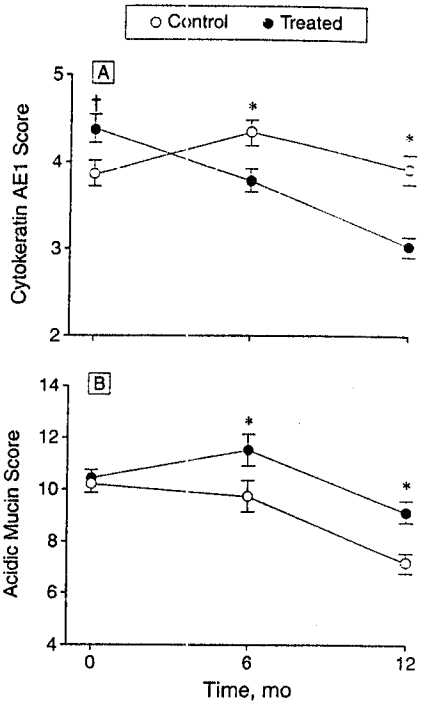

Figure 3.—Effect of dairy supplementation on cytokeratin AE1 and mucins expressions determined in colorectal mucosa. Data shown as mean (SE) arbitrary units in the number of subjects studied at each time point. Asterisk indicates $P<.05$ for time between groups; dagger, baseline levels by Student t test.

Table 4.—Effect of Dairy Calcium Treatment on the Expression of Differentiation Markers in the Colonic Crypt

Marker	Control, Mean (SE)	Treatment, Mean (SE)	%	*P* Value
Cytokeratin AE 1				
Baseline	3.89 (0.15)	4.39 (0.16)	+12.9	.02
6 mo	4.36 (0.14)	3.81 (0.14)	−12.6	<.05
12 mo	3.97 (0.16)	3.06 (0.11)	−22.9	<.05
Acidic mucins*				
Baseline	10.25 (0.5)	10.52 (0.3)	+2.6	>.10
6 mo	9.78 (0.6)	11.61 (0.6)	+18.7	<.05
12 mo	7.23 (0.4)	9.20 (0.4)	+27.2	<.02

*Units represent the relative content of sulphomucin and sialomucin.

Table 5.—Nuclear Morphometric Measurements

	Control, Mean (SE)			Treatment, Mean (SE)		
Variable	Baseline	6 mo	12 mo	Baseline	6 mo	12 mo
Nuclear area, μm²	16.78 (0.36)	17.99 (0.59)	16.80 (0.45)	17.45 (0.38)	16.50 (0.40)*	17.49 (0.39)
Maximal diameter, μm	7.70 (0.10)	7.93 (0.01)	7.77 (0.11)	7.77 (0.10)	7.52 (0.13)*	7.97 (0.12)
Minimal diameter, μm	3.24 (0.05)	3.36 (0.08)	3.20 (0.04)	3.34 (0.04)	3.27 (0.04)	3.24 (0.04)
Perimeter, μm	19.97 (0.26)	20.71 (0.31)	19.95 (0.30)	20.29 (0.26)	19.86 (0.27)*	20.40 (0.28)

*$P<.05$ compared with the control group by Student t test.

in compartment 5. At 12 months in the experimental group, proliferative activity also was decreased throughout the crypt and phi h was less compared with controls (Table 3, Figure 2). The group-by-time interaction from baseline to 12 months also showed a significant reduction in labeled cells per crypt column and in labeling index in the whole crypt in the experimental group compared with controls.

Cytokeratin AE1 expression in the colorectal mucosa was slightly higher at baseline in the experimental group ($P = .02$); in control subjects there were no changes from baseline in the 6- and 12-month biopsy specimens (Table 4). In contrast, the experimental group showed significantly reduced cytokeratin AE1 expression of 13% and 23% at 6 and 12 months, respectively (Figure 3, A), with localization of cytokeratin AE1 near the luminal surface of the colonic mucin as occurs in a normal colon.[14,15] There also was a significant group vs time difference between groups ($P<.01$).

Acidic-mucin immunoreactivity showed no significant differences at baseline between the 2 groups and did not change significantly in controls during the study. However, there was a significant reduction in acidic-mucin scores at 6 to 12 months in the experimental subjects (Table 4, Figure 3, B).[14,16]

Nuclear morphometric measurements showed no differences between the 2 study groups at baseline. Nuclear area, maximum diameter, and perimeter were all significantly lower in the experimental group than in controls at 6 months, but this difference was not maintained at 12 months (Table 5). Changes occurred principally in cells near the base of rectal crypts where cells proliferate in the cell cycle.

The significance of changes in biomarkers in experimental subjects was not reduced when dietary fat was covaried out of the analysis, suggesting that changes in consumption did not contribute to the differences between groups.

COMMENT

The diet is believed to be a major etiologic factor in the development of colo-

rectal cancer.[17] Protective effects of calcium against colonic carcinogenesis have been shown by numerous animal studies.[5] Free bile acids and fatty acids stimulate cell proliferation and irritate or damage colonic epithelial cells. Calcium binds these substances into insoluble complexes, reducing their ability to modify colonic cell proliferation and differentiation.[6,7] Calcium also directly decreases proliferation of colonic and other epithelial cells in vitro and can induce terminal differentiation.[2] Most dietary calcium and vitamin D comes from the ingestion of milk and milk products, and limited studies suggest that calcium from nonfat dried milk may protect against colon carcinogenesis in rats.[18]

In control subjects, food intake remained quite constant so that consumption of total energy, protein, fat, calcium, phosphate, vitamin D, and several other micronutrients did not change significantly. However, subjects increased calcium by a mean of 872 mg, bringing total intake close to 1500 mg/d by means of dairy food supplementation. At baseline, protein and fat intakes in both groups of subjects were similar. Only protein intake increased modestly with dairy food supplementation at 6 months.

In this study, the experimental group of subjects showed consistent changes in cell proliferation kinetics at 6 and 12 months. These findings are similar to several randomized studies of elemental calcium supplementation in adenoma patients.[5,19,20] However, not all studies of calcium supplementation have shown consistent changes.[5] Dairy foods contain significant levels of other components that have decreased the number of neoplasms in animal models,[18] including vitamin D,[9] butyrate,[21,22] sphingomyelin,[23] and conjugated linoleic acid.[24] The cytotoxicity of fecal water also has been shown to be reduced by dairy products[25] and fecal diacylglycerol concentrations decreased during calcium supplementation.[26] Dairy products also have been shown to be antimutagenic by the Ames test.[27]

We also examined a series of biomarkers that identify proliferative activity of epithelial cells and properties associated with their differentiation and maturation (ie, cytokeratins and acidic mucins). Proliferative activity of normal-appearing colonic epithelial cells has previously been associated with risk of colon cancer.[28,29] Cytokeratins are structural proteins of epithelial cells that become modified during cell differentiation, maturation, and transformation.[30-33] Cytokeratin AE1 expression in normal colonic mucosa is confined to the surface epithelium and upper crypt, and cells in the lower crypt are unreactive.[14,15] In contrast, in flat colonic mucosa of patients with familial polyposis and in colonic adenomas and carcinomas, cytokeratin AE1 reactivity expanded throughout the crypt. In our study, flat colonic mucosa at baseline also had strong cytokeratin AE1 reactivity, which extended to the bottom of crypts and is characteristic of diseased mucosa. However, with dairy food supplements, cytokeratin AE1 decreased and localized to the upper crypt as in normal mucosa.

Glycoproteins such as mucins are important in maintaining cell structure and function. Glycoconjugate modifications occur early in colorectal carcinogenesis,[34] and mucins are aberrantly expressed in cancer.[35] In the normal distal colonic mucosa of humans and rodents, sulphomucins are the predominant acidic mucins, and sialomucins are undetectable or present in low amounts.[14,16] In contrast, sialomucins in epithelial cells of adenomas and adenocarcinomas increase and thus the ratio of sulphomucins to sialomucins decreases in these tumors compared with a normal colon.[16] In our study, before the administration of dairy foods, flat colonic mucosa contained abnormally increased sialomucin levels, and after dairy food administration, they decreased, causing the ratio of sulphomucins to sialomucins to increase toward that of normal mucosa.

Computer-assisted cell image analysis provides a method with which to quantitate nuclear morphometric characteristics. Nuclear size has been found to increase in colonic precancerous epithelial cells and in tumor tissue compared with normal colonic cells.[36-38] In our study, nuclear size decreased after 6 months of treatment but appeared unchanged at 12 months. This study is the first to evaluate nuclear histomorphometric changes in rectal biopsy specimens of humans with an intervention regimen using computer-assisted cell image analysis.

The dietary habits of our study subjects who knew they were at increased risk for colon cancer revealed that baseline mean daily energy levels were low, at only about 7140 kJ, comprising 27% of total calories as fat, a calcium intake of 655 mg/d, and a vitamin D intake of 202 IU/d. These values differ from those of the average US population[39] in calories, fat, calcium, and dietary vitamin D content and are closer to those of a health-conscious population.

Thus, the present study reveals that the addition of low-fat dairy food to the diets of subjects at increased risk for colon cancer decreased colonic epithelial cell proliferation and increased epithelial cells with normal differentiation and maturation properties in colonic mucosa. The major nutrient alteration identified was increasing dietary calcium intake from approximately 600 to 700 mg/d to 1500 mg/d. Preliminary data indicate that recurrence in adenomas also are reduced by supplemental calcium administration.[40] The present study did not evaluate whether the treatment altered colonic adenoma recurrence since the number of subjects was too small and the length of the study too short to determine such an effect. The data also does not prove that the calcium content of dairy foods alone was responsible for the biomarker changes observed.

A National Institutes of Health consensus conference evaluating calcium requirements to reduce the risk of osteoporosis has recommended a daily intake of 1200 to 1500 mg of calcium for adults aged 65 years and older.[41] Our experimental group (average age, 66-67 years) received a total daily calcium intake of about 1500 mg and demonstrated findings compatible with risk reduction for colon neoplasia. Our results suggest that supplementing dietary calcium may be beneficial not only for reducing the risk of osteoporosis, but also as a chemopreventive measure for colonic neoplasia.

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