Colonic Epithelial Cell Proliferation Decreases with Increasing Levels of Serum 25-Hydroxy Vitamin D[1]

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Abstract

Epidemiological evidence suggests a potential role for vitamin D in colon cancer prevention. Vitamin D, absorbed from the intestine or derived from solar ultraviolet light, is metabolized in the liver to 25-hydroxyvitamin D (25-OH D_3). Previous studies examining effects of vitamin D upon carcinogenesis have focused upon the active metabolite 1,25-dihydroxyvitamin D [1,25-$(OH)_2$ D_3], which interacts with nuclear vitamin D receptors in several organs. Until recently, the metabolism of 25-OH D_3 to 1,25-$(OH)_2$ D_3 was believed to occur only in the kidney, but more recent studies have shown that 25-OH D_3 conversion to 1,25-$(OH)_2$ D_3 can occur in other tissues. We examined the association between fasting levels of 25-OH D_3, 1,25-$(OH)_2$ D_3, and *Bsm*I polymorphism of the *vitamin D receptor* (*VDR*) gene with indices of colonic epithelial cell proliferation and differentiation in a chemoprevention study, after giving vitamin D or calcium and taking rectal biopsies that were incubated with bromodeoxyuridine. Vitamin D receptor polymorphism was determined by genotyping of the 3′ *Bsm*I polymorphism in intron eight of the *VDR* gene.

No significant changes in cell proliferation or in differentiation were found in subjects between study start and end. However, fasting serum levels of 25-OH D_3 showed a highly significant decrease with whole crypt labeling index and the size of the proliferative compartment (phi h). There was no correlation between serum levels of 1,25-$(OH)_2$ D_3 and the proliferative parameters. Calcium supplementation induced a significant effect upon the relationship between serum 25-OH D_3 and rectal epithelial cell labeling index and phi h when studied by covariance analysis without a relationship with 1,25-$(OH)_2$ D_3 levels. *VDR* genotype did not influence the effects of serum 25-OH D_3 or serum 1,25-$(OH)_2$ D_3 levels upon proliferation.

These data suggest that there might be a local effect of 25-OH D_3 on colonic epithelial cells through conversion of 25-OH D_3 to 1,25-$(OH)_2$ D_3. Subsequent studies have demonstrated the presence of 1α-hydroxylase mRNA in normal colorectal epithelium and in colorectal cancer. Thus, vitamin D may have an important role in determining the effects of calcium on colorectal epithelial proliferation and may explain some of the discrepancies found previously in studies that examine the direct role of calcium on the colorectal epithelium.

Introduction

Epidemiological evidence suggests a potential role of vitamin D in colon cancer prevention. Colon cancer mortality rates show a marked North to South gradient in the United States with higher rates observed in the North (1). Because this gradient correlates with sunshine exposure and therefore levels of ultraviolet radiation, it has been suggested that low ultraviolet exposure may be a risk factor for colorectal cancer (2). Data from nine population-based cancer registries obtained from the Surveillance, Epidemiology, and End Results program also showed an inverse relationship between amounts of solar radiation and colon cancer incidence rates in males (3). Much of an individual's vitamin D is formed in the skin in response to ultraviolet radiation (4).

Dietary vitamin D intake also has been inversely correlated with colon cancer (5, 6) and with colonic adenoma (7) risk. The vitamin D made in the skin as well as that absorbed from the gastrointestinal tract subsequently is metabolized in the liver to 25-hydroxyvitamin D_3. Fasting serum levels of 25-OH D_3[8] generally reflect the vitamin D stores of the body (8). Although the major source of circulating vitamin D is 25 OH D_3, it generally is accepted that 1,25-$(OH)_2$ D_3 is the active metabolite functioning through its interaction with a nuclear 1,25-$(OH)_2$ D_3 receptor (9). The kidney is the major source of 1,25-$(OH)_2$ D_3 through the action of an enzyme, 25-hydroxyvitamin 1$\alpha\alpha$-OHase, although

Received 4/20/01; revised 11/2/01; accepted 11/8/01.
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[1] This study was supported in part by National Cancer Institute Grant 25439-02 and the Ann Fisher Nutrition Fund.
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[8] The abbreviations used are: 25-OH D_3, 25-hydroxyvitamin D_3; 1,25-$(OH)_2$ D_3, 1,25-dihydroxy vitamin D_3; VDR, vitamin D receptor; 1$\alpha\alpha$-OHase, $\alpha\alpha$1-hydroxylase.

Table 1 Fasting serum calcium and vitamin D concentrations[a]

Study subjects	Treatment, daily intake	Serum concentrations		
		Calcium mg/dl	25-OH D_2 ng/ml	1,25-$(OH)_2$ D_3 pg/ml
Group A ($n = 14$)	1500 mg $CaCO_3$	9.47 ± 0.1	26.2 ± 2.5	30.8 ± 2.5
Group B ($n = 12$)	1500 mg $CaCO_3$ 400 IU Vitamin D	9.52 ± 0.1	37.3[b] ± 3.7	27.4 ± 2.4
Group C ($n = 13$)	0.5 μg 1,25$(OH)_2D_3$	9.59 ± 0.09	22.0 ± 1.9	39.8[b] ± 2.3[b]

[a] Fasting serum taken at the conclusion of a 6-month chemoprevention study in the number (*n*) of subjects in each group.
[b] Mean data significantly different from data obtained at study start ($P < 0.05$).

Table 2 Proliferation and vitamin D receptor genotypes

VDR	Labeling index[a] (%)		phi h[a] ($\times 10^{-3}$)	
	Baseline	Study end	Baseline	Study end
BB $n = 10$	6.41 ± 0.5	5.5 ± 0.3	9.31 ± 2.8	9.87 ± 2.2
Bb $n = 19$	5.32 ± 0.3	5.83 ± 0.2	9.1 ± 1.7	7.4 ± 0.7
bb $n = 11$	6.28 ± 0.5	5.78 ± 0.4	7.32 ± 1.0	4.65 ± 1.0

[a] Data are presented as mean ± SE in the number (*n*) of subjects having each genotype. There were no significant differences among the three groups of subjects.

nonrenal sources of the enzyme have been reported recently in macrophages (10), keratinocytes (11), and the prostate (12). Prostatic cells, like many other tissues (8), demonstrate the presence of 1,25-OH_2 D_3 receptors (13).

In the present study, we have examined relationships between fasting levels of 25-OH D_3 and 1,25-$(OH)_2$ D_3 in the serum with indices of rectal epithelial cell proliferation and differentiation. Observations were made in the context of a chemoprevention study that measured effects of vitamin D and calcium on epithelial cells in the colon. The results of this study clearly demonstrate highly significant correlations between increased levels of circulating 25-OH D_3 and several independent indices of cell proliferation, which have been associated with a lower risk for colorectal neoplasia.[9] The findings further document the importance of serum 25-OH D_3 levels in the previously observed beneficial role of calcium in inhibiting colonic epithelial cell proliferation and thereby a putative risk of colorectal neoplasia.

Materials and Methods

The data presented here were obtained in a prospective blinded randomized study of the administration of either calcium, calcium plus supplemental dietary vitamin D_3, or 1,25-OH D_3 for 6 months, upon indices of rectal epithelial cell proliferation and differentiation in subjects at increased risk for colonic neoplasia. Potential subjects were chosen from the endoscopic and pathology records of St. Luke's-Roosevelt Hospital Center and Columbia Presbyterian Medical Center, both university hospitals of the College of Physicians & Surgeons of Columbia University in New York City. Eligible subjects had a resected pathologically documented colorectal adenomatous polyp removed within 3 years of entering the study. Excluded were patients with a history of familial cancer syndromes, with a personal history of cancer other than nonmelanoma skin cancer, intestinal malabsorption, inflammatory bowel disease, or prior gastrointestinal surgery except appendectomy. Further exclusions were known abnormalities of calcium metabolism, hyperthyroidism or history of milk-alkali syndrome, renal stones, or taking steroid-binding resins. Subjects also were not permitted to have taken >660 mg/day of aspirin or usual daily dosages of nonsteroidal anti-inflammatory drugs within the previous 3 months, or be taking other investigational drugs or medications that might interfere with the study end points. Furthermore, dietary exclusions included the ingestion of more than a total of 1000 mg of calcium or 200 mg of supplemental calcium per day or vitamin D >200 IU/day. Exclusions based upon serological determinations included hypercalcemia (serum level >10.6 mg/dl), current hyperphosphatemia (serum level >6.0 mg/dl), or evidence of significant renal insufficiency (blood urea nitrogen >1.5 times normal).

Subjects were randomized to one of three chemopreventive arms: group A, 1250 mg calcium carbonate equivalent to 500 mg of elemental calcium three times daily; group B, 1250 mg calcium carbonate (500 mg of elemental calcium) orally three times daily with 400 IU vitamin D_3 orally daily; or group C, 0.25 μg 1,25-$(OH)_2$ D_3 orally twice daily (Table 1) for 6 months. The study was approved by the Institutional Review Boards of both institutions, and all subjects gave informed consent for the study.

At the beginning of the study (baseline) and at study end, rectal biopsies and vitamin D measurements were obtained. Flexible sigmoidoscopy and rectal biopsies were performed between 8 and 11 a.m. in the majority of subjects and between 1 and 2 p.m. in some subjects (at the end of the study, the biopsies in the same individuals were taken at identical times of the day). Sigmoidoscopy was performed without analgesia; the rectal mucosa was examined to 20–25 cm to exclude any inflammation or recurrent polyps. Six to eight biopsies of normal-appearing rectal mucosa were taken (~2 in each quadrant) 10–15 cm from the anal verge. Biopsies were placed in a container with MEM containing bromodeoxyuridine (0.1 mg/ml), cut into small pieces, and flattened on a piece of Metrocell filter. The biopsies then were incubated in a Bellco gas chamber with 95% O_2, 5% CO_2 and placed in a rocking platform at 10 cycles/minute at 37°C for 1 h. The tissues then were transferred into 95% ethanol and fixed overnight; the fixative was changed once the next day, and then specimens remained in the fixative until processed by standard methods for embedding in paraffin. Three-μm-thick serial tissue sections were prepared for immunohistochemical studies. The sections were organized in ribbons to avoid studying the same crypt more than once.

At baseline and study end, ~10 ml of blood were drawn; serum was separated and then was assayed for calcium, phosphate, creatinine, blood urea nitrogen, and albumin. A tube, protected from light, was analyzed for 25-OH and 1,25-$(OH)_2$ D_3 levels by a radio receptor method using a Nichols Institute assay kit (15, 16).

End Point Measurements. The proliferative kinetics of the rectal mucosa were determined by measuring the relative in-

[9] Presented as an abstract during Digestive Disease Week 2000 (14).

Table 3 Correlation between colonic epithelial proliferation and serum vitamin D_3 levels

Total group	Baseline				Study end			
	Proliferation	Slope	r^2	*P*	Proliferation	Slope	r^2	*P*
25-OH D_3								
Epithelial cell	56.1 ± 5.0	+0.06	0.102	0.437	57.6 ± 5.7	+0.08	0.027	0.337
Labeled cell	3.24 ± 0.9	−0.01	0.024	0.346	3.28 ± 0.6	−0.22	0.165	0.014
Labeling index %	5.79 ± 1.6	−0.03	0.040	0.226	5.73 ± 1.2	−0.45	0.208	0.005
phi h × 10^{-2}	8.22 ± 7.	+0.96	0.024	0.346	6.98 ± 4.8	−0.14	0.120	0.038
Lh × 10^{-1}	2.79 ± 2.67	+0.16	0.005	0.684	2.48 ± 2.6	−0.67	0.160	0.016
1,25-$(OH)_2$ D_3								
Epithelial cell	56.1 ± 5.0	+0.03	0.003	0.737	57.6 ± 5.67	+0.16	0.084	0.087
Labeled cell	3.24 ± 0.94	+0.01	0.006	0.654	3.28 ± 0.62	+0.02	0.093	0.070
Labeling index	5.80 ± 1.6	+0.01	0.005	0.666	5.73 ± 1.16	+0.02	0.028	0.335
phi h × 10^{-2}	8.22 ± 7.02	−0.03	0.002	0.787	6.98 ± 4.81	+0.09	0.036	0.277
Lh × 10^{-1}	2.79 ± 2.69	+0.06	0.001	0.885	2.48 ± 2.59	+0.47	0.062	0.144

Table 4 Correlations between AE1 cytokeratin and acidic mucin expression and serum vitamin D_3 levels at study end

	Cytokeratin AE1		Acidic mucins	
	r^2	*P*	r^2	*P*
25-OH D_3				
Group A + B + C	0.008	0.586	0.010	0.437
Group A + B	0.000	0.942	0.003	0.824
Group A	0.387	0.018	0.012	0.416
1,25-$(OH)_2$ D_3				
Group A + B + C	0.102	0.047	0.035	0.201
Group A + B	0.049	0.279	0.075	0.163
Group A	0.092	0.293	0.100	0.248

corporation of bromodeoxyuridine into nuclear DNA (16). Twenty half-crypt columns or more were recorded from each biopsy for individual measurements. Crypt columns were measured as the crypt lumen visible from the opening to the base, lined up with one layer of cells. The numbers of cells and the number of labeled cells in each half crypt were determined from the midpoint between two crypts at the surface to the midpoint of the deepest part of the crypt. The labeling index of the total crypt was determined from the number of labeled cells divided by the total number of cells in the crypt. The changes in the distribution of proliferating cells within the rectal mucosa also were determined and analyzed by reference to five equal zones along the rectal crypt from mouth to base (17). A primary end point for proliferative cell distribution was a determination of phi h, which represents the percentage of proliferating (labeled) cells in the upper 40% of the crypt as a ratio of the total number of labeled cells in the crypt. Preliminary data in this laboratory using two separate biopsies from the same subject showed that the within-subject variation was small (~15%).

Indices of cell differentiation that were analyzed were cytokeratin AE1 (18) and acidic mucins (18). AE1 expression was demonstrated immunohistochemically using the avidin-biotin-peroxidase technique. The monoclonal antibody to AE1 that was used was purchased from Signet Laboratories, Inc. (Dedham, MA). A semiquantitative scoring method was used to evaluate AE1 expression based on the positive area of staining. To study acid mucins cytochemically, a high-iron diamine-Alcian blue stain was used. With this technique, sulfomucins and sialomucins presented in different colors in the cytoplasm. A semiquantitative measurement was used to evaluate the ratio of number of cells with sulfomucin to sialomucin. These markers had been determined by immunohistochemical methods previously for evaluating the effects of potential chemopreventive agents upon cell differentiation properties (18).

Vitamin D receptor polymorphism was determined on DNA extracted from whole blood. Genotyping of the 3′ *Bsm*I polymorphism in intron 8 of the *VDR* gene was performed by an established method (19) in the laboratory of Dr. Katherine A. McGlynn.

Statistical Methods. To ensure that no differences existed between groups prior to randomization, an ANOVA on the group effect was performed on baseline means for each dependent variable. Then the effects of the group assignment on each dependent variable were analyzed in two ways: (*a*) mean change in each dependent variable was calculated and tested for significance by paired *t* test for each group separately and for all three groups combined to determine whether any variables changed significantly from baseline; and (*b*) the mean change in each dependent variable was compared between groups by means of ANOVA. The results of statistical analyses are described as statistically significant when $P < 0.05$. Levels of serum of 25-OH D_3 and 1,25-$(OH)_2$ D_3 were further correlated with individual indices of cell proliferation and differentiation. Correlation and linear regression studies were further performed with a MiniTab statistics program to correlate the serum levels of 25-OH D_3 and 1,25-$(OH)_2$ D_3 with cell proliferation and differentiation measurements.

To determine whether calcium intake influenced the relationship between labeling indices and phi h and levels of serum 25-OH D_3 and 1,25-$(OH)_2$ D_3, an analysis of covariance was performed with labeling indices and phi h as the dependent variable, calcium treatment as the independent variable, and levels of serum vitamin D_3 as covariates.

Results

The study was started in January 1996, and the last patient completed the study in January 1998. Of the 45 subjects enrolled into the study, there were 6 dropouts; therefore, 39 subjects completed the study. Dropouts were divided similarly between the study groups. There were 22 males and 17 females (mean age, 61.5 years) who completed the study. Twenty-nine subjects were Caucasian, 7 were black, and 2 were Hispanic. The mean overall compliance from pill counts was 86.9% and did not differ amongst the study groups.

The daily intake of dietary components did not differ at baseline or study end among the three groups. Mean calcium intake was ~650 mg/day, and mean dietary vitamin D intake was ~8 μg/day at baseline. The calcium intake of group A subjects from dietary and supplemental sources totaled a mean

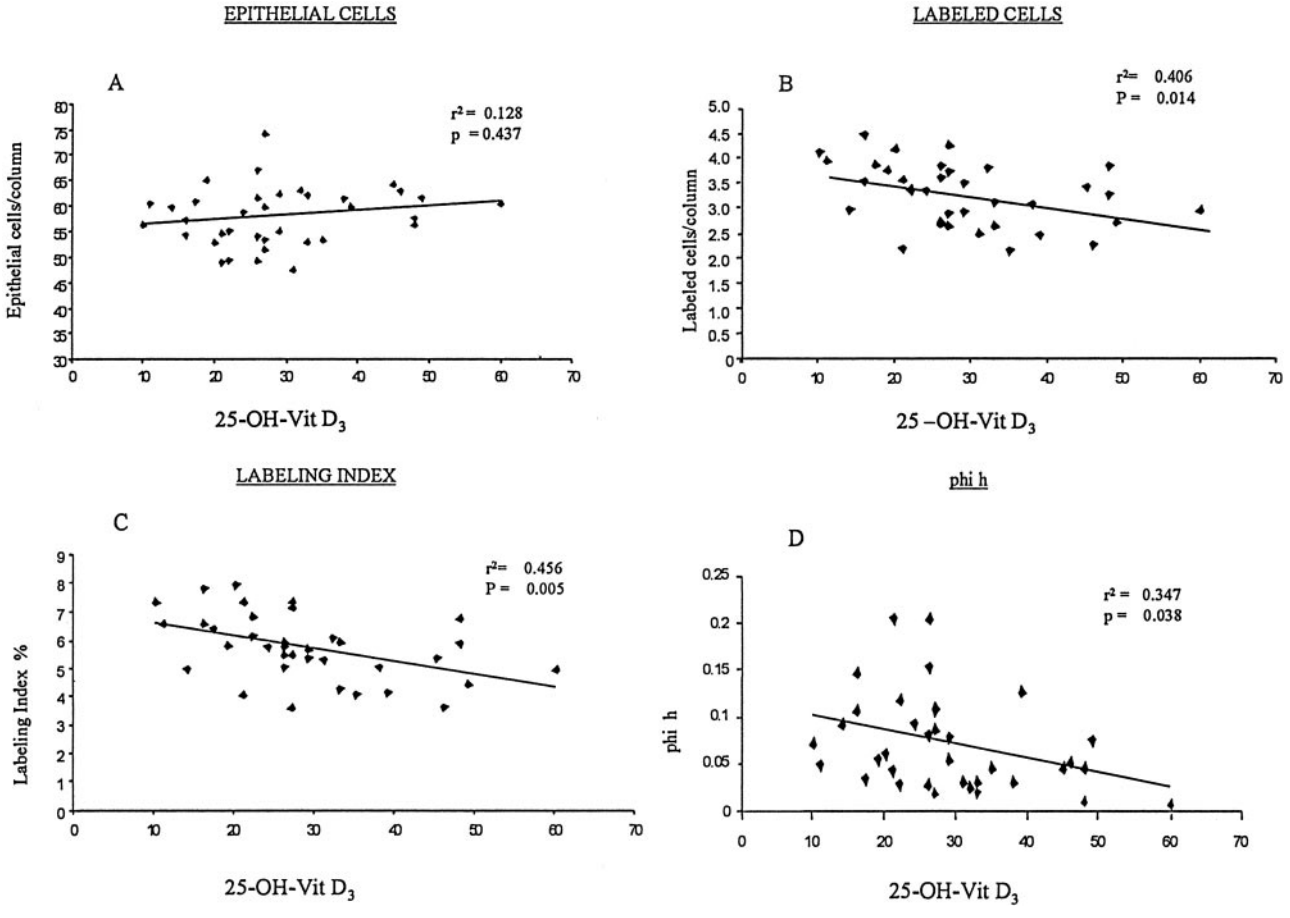

Fig. 1. Correlation between serum levels of 25-OH vitamin D and cell proliferation. Data are shown in ng/ml at study end from the total group of 39 subjects that completed the study. Correlation (r^2) and *P*s shown on each figure. *Top left*, total epithelial cell number per crypt column; *top right*, labeled cells/crypt column; *bottom left*, labeling index (%); *bottom right*, phi h value.

of 1922 mg/day, and group B subjects received a total of 1925 mg/day. The vitamin D intake of group B subjects totaled 19.8 μg/day during the study. Standard laboratory determinations of serum phosphorus, alkaline phosphatase, and liver and renal function were within normal limits and did not change between baseline and study end in any study group.

At baseline, serum levels of calcium, 25-OH D_3, and 1,25-$(OH)_2$ D_3 were within normal limits and did not differ among the groups. Serum calcium and vitamin D concentrations at the completion of the study are shown in Table 1. There were no significant changes in blood levels of 25-OH D_3 nor 1,25-$(OH)_2$ D_3 during the study period, except in group B subjects receiving vitamin D supplements, who increased their 25-OH D_3 level by 44% ($P < 0.001$) without significant changes in 1,25-$(OH)_2$ D_3 levels, and in group C subjects, who received 1,25-$(OH)_2$ D_3 where there were no changes in 25 OH D_3 levels and a modest (14%) but significant rise in serum 1,25-$(OH)_2$ D_3 levels.

The proliferative indices did not change between study start and study end within any group.[10] Furthermore, the VDR *Bsm*I genotypes of the study groups did not affect proliferative indices (as shown in Table 2) for total crypt labeling index and phi h. We then studied the relationship between fasting serum levels of 25-OH D_3 or 1,25-$(OH)_2$ D_3 and crypt cell proliferation kinetics (Table 3) and differentiation markers (Table 4) at the onset and end of the study. At baseline, no correlations were found between the serum levels of either 25-OH or 1,25-$(OH)_2$ D_3 and the proliferation cell kinetics or differentiation markers (cytokeratin AE1 or acidic mucin scores). This may have occurred because there was relatively little variation in the blood levels of serum 25-OH D_3 or 1,25-$(OH)_2$ D_3 metabolites before supplemental calcium or vitamin D was administered. In striking contrast, there were significant correlations between serum 25-OH D_3 levels and epithelial cell proliferative kinetics at the 6-month end point of the study (Fig. 1), whereas no correlation were found between serum 1,25-$(OH)_2$ D_3 vitamin levels and either whole crypt labeling index or phi h (Fig. 2).

To evaluate these correlations at study end more closely, we determined the relationship between proliferative indices and vitamin D levels in subjects in groups A, B, and C separately. In group C subjects receiving 1,25-$(OH)_2$ D_3 for 6 months, there were no significant correlations with any of the individual parameters studied. In contrast, despite the small number of subjects examined, cell proliferation decreased as blood levels of vitamin 25-OH D_3 increased in subjects in groups A and B who were taking supplemental calcium ($P = 0.005$ and 0.08; Fig. 3).

[10] Unpublished data.

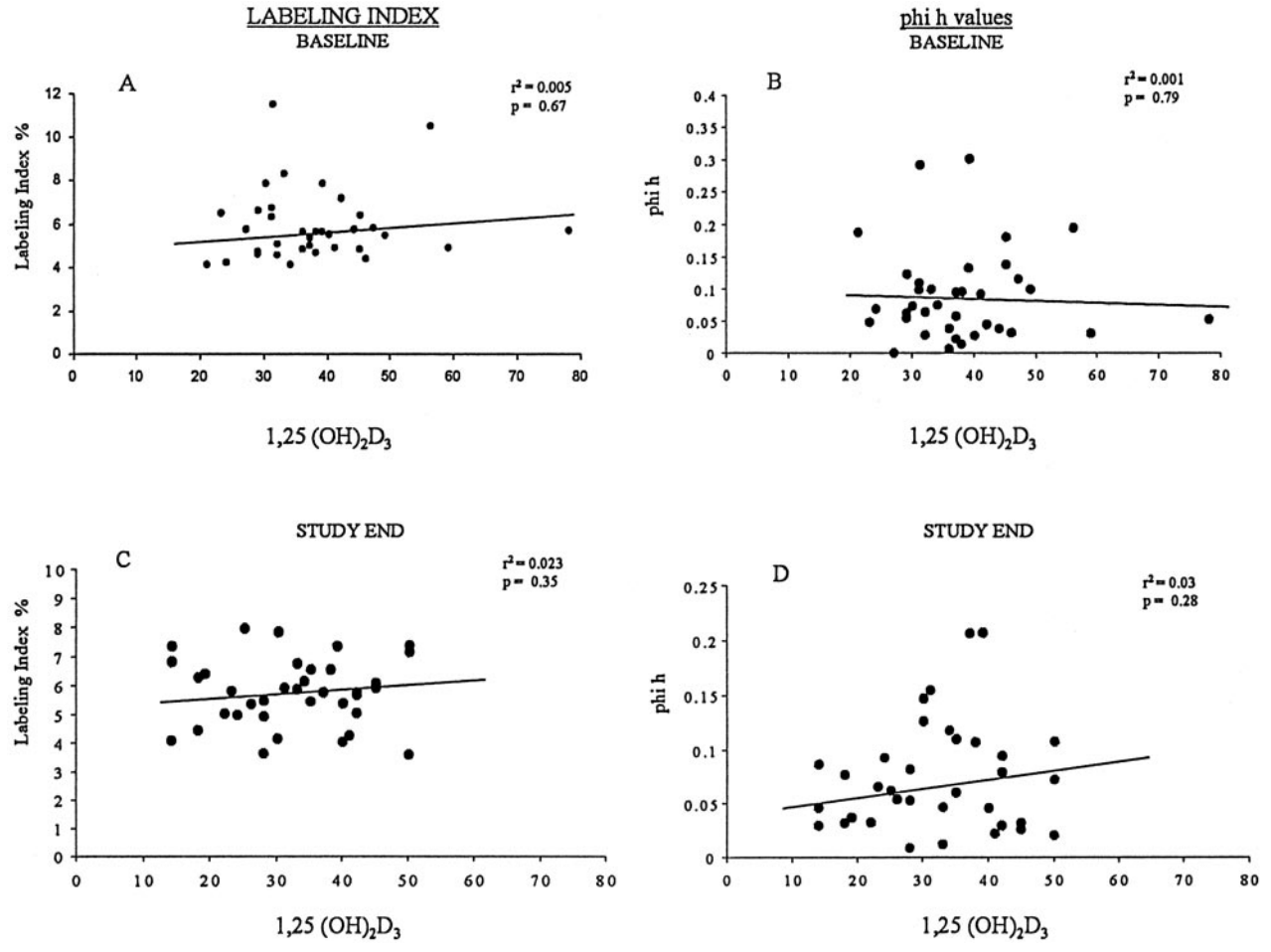

Fig. 2. Correlation between serum levels of 1,25-$(OH)_2$ vitamin D and cell proliferation. Data are shown in pg/ml from the total group of subjects that completed the study. Correlations and *P*s as in Fig. 1. *Top left*, labeling index (%) at baseline; *bottom left*, labeling index (%) at study end; *top right*, phi h at baseline; *bottom right*, phi h at study end.

We then evaluated the effect of calcium supplementation upon the relationships between serum 25-OH D_3 and epithelial cell labeling index and phi h at the study end. This was done using an analysis of covariance for the slopes in groups A + B subjects (who were taking supplemental calcium) and the slope for group C subjects (no supplemental calcium) for 25-OH D_3 with individual parameters of proliferation. There was a significant difference between the slopes for labeling index (significance of covariance, $P = 0.03$) and a trend for phi h ($P = 0.08$). In contrast, there was no effect of calcium upon 1,25-$(OH)_2$ D_3 for either labeling index (significance of covariance, $P = 0.7$) or for phi h ($P = 0.59$). Thus, supplemental calcium increased the effects of 25-OH D_3 upon epithelial cell proliferation.

We further determined relationships between serum 25-OH D_3 and 1,25-$(OH)_2$ D_3 levels with VDR genotypes without finding any significant correlations with proliferation indices. Furthermore, there were no significant correlations between the acidic mucin scores or cytokeratin AE1 scores and serum concentration of either 25-OH D_3 or of 1,25-$(OH)_2$ D_3 (Table 4).

Discussion

In the present prospective randomized study of the effects of the oral administration of calcium and/or vitamin D as calciferol or as 1,25-$(OH)_2$ D_3 on indices of rectal epithelial proliferation and differentiation, we had the opportunity to determine relationships of proliferative parameters to circulating 25-OH D_3 and 1,25-$(OH)_2$ D_3. Vitamin D activity in epithelial cells involves the conversion of 25-OH D_3 to 1,25-$(OH)_2$ D_3 by a 1$\alpha\alpha$ hydroxylase enzyme and the interaction of 1,25-$(OH)_2$ D_3 with nuclear receptors. These receptors are a thousand-fold more responsive to 1,25-$(OH)_2$ D_3 than to 25-OH D_3 and determine vitamin D action on a variety of end organs. Vitamin D is well known to have profound effects upon small intestinal cells upregulating calbindin, the cytosolic calcium binding protein (20), as well as calcium pumps situated on the lateral basolateral membrane of small intestinal epithelial cells (21). These cellular effects are believed to greatly enhance the transport of calcium from the lumen of the small intestine and colon into the bloodstream (22, 23). Because the colon is also a site for calcium absorption, it has been assumed that 1,25-$(OH)_2$ D_3 similarly affects cellular transport in colonic epithelial cells. Furthermore, until recently, it has been believed that virtually all 1,25-$(OH)_2$ D_3 is formed from 25-OH D_3 in the kidney, based upon the observation that nephrectomy almost completely depletes circulating 1,25-$(OH)_2$ D_3 (24).

However, in pathological states, such as sarcoidosis, the body is known to be very sensitive to administration of calciferol (25).

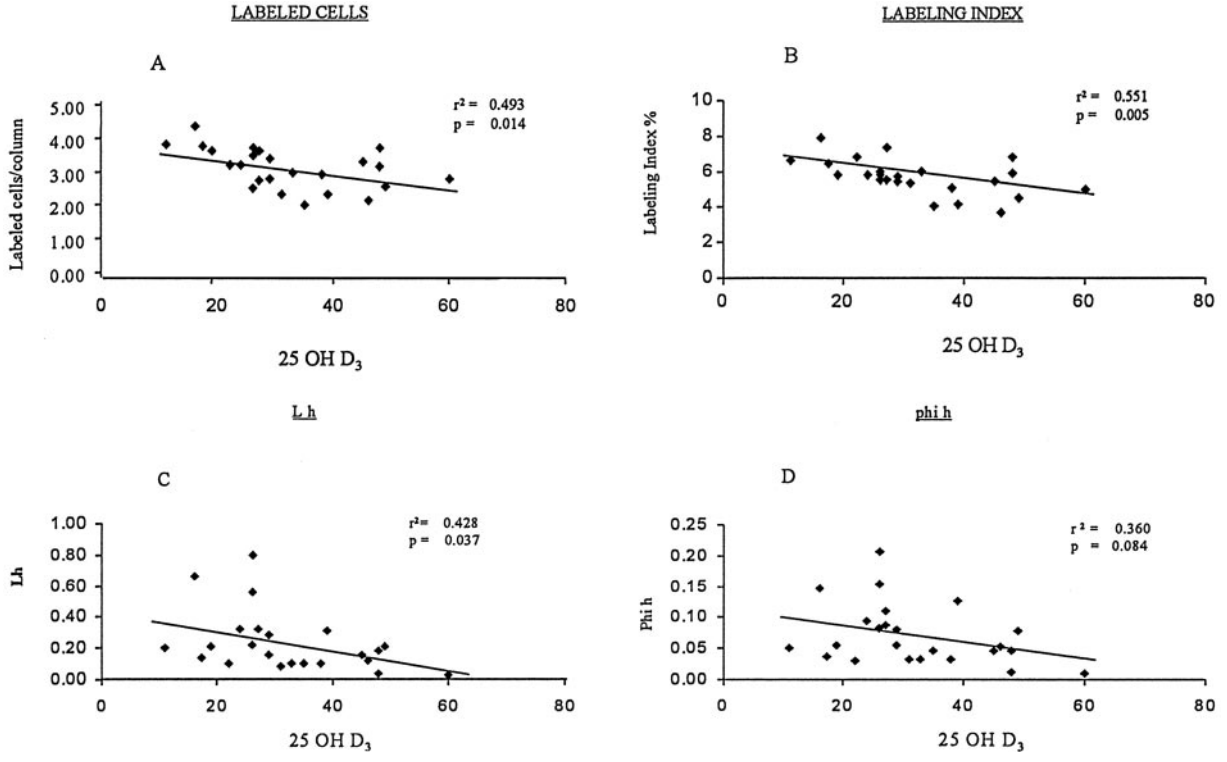

Fig. 3. Correlation between serum levels of 25-OH vitamin D and cell proliferation. Data are shown in ng/ml at study end from subjects in groups A + B given calcium ± vitamin D who completed the study. Correlations and *P*s as in Fig. 1. *Top left*, labeled cells/crypt; *top right*, labeling index (%); *bottom left*, Lh value; *bottom right*, phi h value.

This has been shown to result from formation of 1,25-$(OH)_2$ D_3 in monocytes because of the presence in these cells of a 25-hydroxyvitamin D 1$\alpha\alpha$-OHase (26, 27). Very recently, extrarenal conversion of 25-OH D_3 to 1,25-OH_2 D_3 has been demonstrated to occur through the action of such a 1$\alpha\alpha$-OHase enzyme in keratinocytes (11) and prostatic cancer cells *in vitro* (12). Furthermore, prostatic cells have been shown to have VDRs that respond to the addition of 1,25-$(OH)_2$ D_3 to an incubation medium (14). It has been proposed very recently that vitamin D may be a chemopreventive or chemotherapeutic agent for prostate cancer.

VDRs have been demonstrated to be present in colon cancer cells (28, 29). Such colon cancer cells respond to incubation with 1,25-$(OH)_2$ D_3 at concentrations between 1×10^{-9} and 1×10^{-7} M by inhibition of proliferation (30, 31) and, in some situations, enhanced differentiation (32). Furthermore, administration of 1,25-$(OH)_2$ D_3 to experimental animals deficient in vitamin D has resulted in a series of different cellular changes within the colon (33–35). Because of these observations, it has been suggested that noncalcemic derivatives of 1,25-$(OH)_2$ D_3, which can interact with colonic VDRs, might be useful in the treatment or prevention of colorectal neoplasia (36).

The present study clearly shows significant correlations between circulating levels of 25-OH D_3 with indices of colorectal epithelial cell proliferation. These observations led us to suggest that there might be 1$\alpha\alpha$-OHase enzyme present in normal colonic mucosa that could convert circulating 25-OH D_3 to 1,25-$(OH)_2$ D_3 in epithelial cells. Indeed, very recent preliminary observations in our laboratory have demonstrated the presence of the mRNA 1$\alpha\alpha$-OHase in extracts of normal colorectal mucosa and greatly increased levels in some adjacent human colon cancers (37). Since the completion of this report, Zehnder *et al.* (38) have described extrarenal 1$\alpha\alpha$-OHase in several tumors including the colorectum using *in situ* hybridization and immunohistochemical analysis. Cross *et al.* (39) also have demonstrated very recently 1$\alpha\alpha$-OHase mRNA in colonic tumors and normal adjacent colonic tissue. It therefore now appears that there can be local autocrine or paracrine control of 1,25-OH_2 D_3 formation from 25-OH D_3 in colorectal epithelia, which thereby alters colonic cell proliferation.

Our data also suggest that there is an important interaction between administration of supplemental dietary calcium and the levels of circulating 25-OH D_3, which has an effect upon colorectal epithelial cell proliferation. Thus, some of the variation in colorectal cell proliferation occurring after calcium administration reported in several previous studies (40–42) might have been attributable to lower serum 25-OH vitamin D levels. Vitamin D may have a further important role in mediating a calcium inhibitory effect on abnormal colorectal cell development (43, 44) and in determining an intracellular calcium gradient in mammalian colonic crypts (45). Future studies of the chemopreventive action of calcium and vitamin D must take into account the levels of circulating 25-OH D_3 and probably the activity of the colonic enzyme that converts 25-OH D_3 to 1,25-$(OH)_2$ D_3.

Acknowledgments

We thank Dr. John O'Connor for performing the vitamin D assays, Ann Washington for excellent technical assistance, Jodi Gilman for nutritional evaluation of the subjects, and Dr. Donald McMahon for data management. Furthermore, we thank the many physicians who allowed their patients to be studied and, above all, the forbearance of the volunteer subjects.

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Correction

Re: Holt, *et al:* Colonic Epithelial Cell Proliferation Decreases with Increasing Levels of Serum 25-Hydroxy Vitamin D. *11:* 113–119, January 2002.

Dr. Kurihara's initials would also be listed with Strang Cancer Prevention Center, New York, New York. The authors names should read as follows:

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Re: Dietrich, *et al:* Antioxidant Supplementation Decreases Lipid Peroxidation Biomarker F_2-isoprostanes in Plasma of Smokers. *11:* 7–13, 2002.

Footnote 1 on page 7 should have included the following:

Supported by the University of California Tobacco-Related Disease Research Program (TRDRP:6RT-0008 and 7RT-0160), by the NIH Grant P30 ES01896, and in part by the German Academic Exchange Service, DAAD, Postdoctoral Fellowship for Dr. Marion Dietrich. Work in J. D. M.'s laboratory is supported by NIH Grants GM15431, DK26657, and DK48831. J. D. M. is the recipient of a Burroughs Wellcome Fund Clinical Scientist Award in Translational Research.

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Cancer Epidemiol Biomarkers Prev 2002;11:113-119.