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Serum levels of vitamin D metabolites and the subsequent risk of colon and rectal cancer in Finnish men

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Experimental and human epidemiologic data suggest a protective role for vitamin D in large bowel cancer. To investigate this association, we conducted a nested case-control study within a Finnish clinical trial cohort. Cases (n = 146) were participants diagnosed with primary adenocarcinoma of the large bowel. Controls were matched (2:1) to cases on age, date of baseline blood draw, and study clinic. Prediagnostic serum levels of the vitamin D metabolites, 25-hydroxyvitamin D (25-OH D), and 1,25-dihydroxyvitamin D (1,25-DIOH D) were used as primary exposure measures. The baseline geometric-mean serum level of 25-OH D was 11.6 percent lower in cases than in controls (12.2 *cf* 13.8 ug/l, P = 0.01) while serum levels of 1,25-DIOH D did not differ by case-control status. No association was seen between serum levels of 1,25-DIOH D and large bowel cancer risk. However, the estimated relative risk (RR) of large bowel cancer decreased with increasing level of serum 25-OH D and the association was more pronounced for rectal cancer (55 cases; RR by quartile = 1.00, 0.93, 0.77, 0.37; trend P = 0.06). Neither exclusion of early cases nor multivariate adjustment for potential confounders materially altered these estimates. There was no evidence of effect modification by level of 1,25-dihydroxyvitamin D or with other known risk-factors for large bowel cancer. *Cancer Causes and Control* 1997, 8, 615-625

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Introduction

Large bowel cancer is a major public health problem in western industrialized countries. In the United States, it is the second leading cause of cancer mortality. In 1994, 149,000 new cases of colorectal cancer were diagnosed and 59,000 people died from the disease in the US.[1] Over a lifetime, approximately one in 15 people will develop colorectal cancer.[2] Within Finland, it is one of the most common cancers and its incidence has been on the rise since the 1950s.[3] Wide international differences in incidence rates and rapid changes in the prevalence of colorectal cancer among migrants to those of their host country point to strong environmental influences in its etiology.

Epidemiologic evidence suggesting a role for vitamin D in large bowel cancer has been available since the early 1980s. Results from ecologic studies support an inverse relation between level of solar radiation and colorectal cancer mortality and incidence.[4-6] Since the major source

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of vitamin D is through sunlight-induced photobiosynthesis in the skin, it was hypothesized that the protective effects observed may be mediated by vitamin D levels. In addition, two large prospective cohort studies[7,8] found an inverse association between vitamin D intake from diet and/or supplements and large bowel cancer risk. Of two nested case-control studies[9,10] conducted within the same cohort examining the relation between prediagnostic serum levels of vitamin D metabolites and colon cancer risk, only one[9] reported an inverse association. Epidemiologic studies supporting the association between vitamin D and colorectal cancer have been reviewed.[11,12]

Since its discovery as a 'nutrient' over 80 years ago, research on vitamin D has been extensive and evolving.[13-18] It was first identified as an essential factor in maintaining calcium homeostasis. Observations that the hormonal form of vitamin D, 1,25-dihydroxyvitamin D (1,25-DIOH D), could both induce the differentiation of mouse myeloid leukemia cells into macrophages[19] and inhibit the growth of a cultured melanoma cell line,[20] along with the discovery of the vitamin D receptor in malignant tissues and several cancer cell lines (osteosarcoma, breast, lung, ovary, colon),[21] stimulated interest in vitamin D's role in carcinogenesis. Since the 1980s, there have been a number of experimental studies examining the effect of vitamin D and several of its synthetic analogs in both reducing the growth of implanted tumor xenografts and inhibiting the formation of chemical carcinogen-induced tumors of the skin, mammary gland and colon. Indeed, a number of *in vitro* and experimental studies provide direct evidence that vitamin D plays a role in large bowel carcinogenesis.[22-30]

The major source of vitamin D (up to 80 percent) in humans is derived from cutaneous synthesis initiated by exposure to the ultraviolet B range of sunlight. Vitamin D is transported in blood predominantly bound to a specific α-globulin, vitamin D binding protein (DBP). Once transported to the liver, it is hydroxylated to its principal circulating form, 25-hydroxyvitamin D (25-OH D), which has an *in vivo* serum half-life of approximately three weeks.[31] Since this reaction is not regulated homeostatically, 25-OH D is not retained in the cells but is released to the circulation where it accumulates by binding to DBP. At normal serum concentrations, only small amounts of 25-OH D are released from this pool to enter tissues. Therefore, serum levels of 25-OH D best reflect the overall vitamin D status of an individual. Due to the effect of cutaneous photobiosynthesis, there is substantial seasonal variation in serum levels of 25-OH D, with the highest levels found in late summer and early autumn.[32-37] The active, hormonal form of the vitamin, 1,25-DIOH D, is formed by hydroxylation of 25-OH D in the kidney[14-16] which is tightly regulated through a complex process to maintain adequate levels of extracellular calcium. Unlike 25-OH D, 1,25-DIOH D is distributed largely intracellularly, where it is present exclusively in the nucleus and cytoplasm bound to a specific protein. As such, levels of 1,25-DIOH D in the circulation are orders of magnitude less than serum 25-OH D. The *in vivo* serum half-life of 1,25-DIOH D is about four to six hours.[38]

Given the abundant *in vitro* and experimental evidence of a role for the hormonal form of vitamin D in both normal and malignant cell proliferation and differentiation and the modest epidemiologic data suggesting a possible protective association between vitamin D status and large bowel cancer risk, we set out to examine this putative association among a cohort of male smokers who participated in a large clinical trial in southwestern Finland.

Materials and methods

Study cohort

Case and control subjects for this study were selected from a cohort of participants in the Alpha-Tocopherol, Beta-carotene Cancer Prevention Study (ATBC Study). The ATBC Study was a randomized, double-blind, placebo-controlled, 2 × 2 factorial design, primary prevention trial, whose principal objective was to test the effects of α-tocopherol (50 mg/day) and β-carotene (20 mg/day) supplements on lung cancer incidence and mortality.[39-40] The trial was conducted between 1985 and 1993 in southwestern Finland as a joint project between the National Public Health Institute of Finland and the US National Cancer Institute. Potential participants were identified from the computer list of the national population registry of Finland. To be eligible, participants had to be male, aged 50 to 69 years, a current smoker of five or more cigarettes per day, a resident within the geographic region of the study, and willing to participate and give informed consent. Reports have been published detailing the ATBC Study's design, methods and participant characteristics [39] as well as its main results. [40]

Selection of cases and controls

Cases were those trial participants who were diagnosed between January 1985 and November 1993 with primary adenocarcinoma of the colon (ICD-9[41]-153, $n = 91$) or rectum (ICD-9-154, $n = 55$). Tumors were distributed throughout the large bowel (43 colon cancer cases proximal to the splenic flexure, 48 colon cancer cases distal to the splenic flexure and 55 cases in the rectosigmoid, rectum, and anal canal). Cases were identified through the Finnish Cancer Registry;[42] their medical records, along with pathology specimens, were reviewed centrally by at least two study physicians, including one medical oncologist. Pathology and cytology specimens were reviewed

by one of the several organ-system-specific pathology review groups, who assigned the final histopathologic diagnosis (World Health Organization [WHO] classification) to the case. In this study, for five cases having more than one malignant colorectal tumor, the histology and diagnosis date for the earliest cancer diagnosed was used.

Using incidence-density sampling,[43] controls were matched 2:1 to cases on age (± 1 year), study clinic, and date of blood draw (± 28 days). Controls had to be alive and free of colon and rectal cancer at the time the case was diagnosed. Nine controls had a diagnosis of another cancer prior to being selected as a control at the time of diagnosis of the matched colorectal case. Elimination of these controls from the analysis did not materially affect case-control differences or risk estimates for the exposures of interest, serum levels of vitamin D metabolites. Therefore, they were included in all subsequent analyses. Serum was collected at the time of randomization (on average three and one-half years prior to case diagnosis; range of one to seven years) and was analyzed in case-control triplet sets. Stored serum was unavailable for two controls selected for two cases; therefore, the total number available for serum assay was 146 cases and 290 controls. As an additional quality-control measure, we included masked reference sera (composed of pooled sera) among case-control samples sent to the laboratory for assay (a total of 10 percent of the samples). Lab personnel were blind to both case status and to the identity of quality-control serum samples. A radioimmunoassay with an I^{125} tracer was used to determine serum levels of 25-OH D[44] and a radioreceptor assay was used for 1,25-DIOH D.[45] The intra-set coefficients of variation (based on masked reference serum assays) were 9.5 percent and 7.5 percent, respectively. The inter-batch coefficients of variation were 13.6 percent and 7.2 percent, respectively.

Information on variables that possibly could confound the association between serum metabolites of vitamin D and colorectal cancer risk were available from ATBC Study baseline questionnaires, medical histories, and physical examinations or measurements. Lifestyle factor information such as education, physical activity, marital status, smoking history and place of residence was available for all clinical trial participants. Dietary information[46] that could relate to colorectal cancer risk (intake of fat, fiber, protein, and calcium) was available on a majority of cases and controls (137 cases; 259 controls). Body mass index (BMI) (kg/m^2) was calculated from baseline measures of height and weight. Data on important predisposing medical conditions (*e.g.*, history of adenomatous polyps) or hereditary risk factors (*e.g.*, family history of colorectal cancer) was not collected at baseline. Baseline measurements of serum α-tocopherol, β-carotene, and cholesterol were available for the entire cohort. Since this was an intervention trial, treatment group assignment also was evaluated as a potential confounder or effect modifier.

Prior reports that the etiology and pathogenesis of colon cancer may differ by anatomic location[47-57] led us to explore the effect of serum 25-OH D on cancer risk by anatomic subsite within the colon (n = 91 cases). Colon cancer cases were divided into proximal colon (n = 43; includes tumors in the cecum, appendix, ascending colon, transverse colon, and hepatic flexure) and distal colon (n = 48; includes tumors in the splenic flexure, descending colon, and the sigmoid colon) and risk estimates for serum 25-OH D were calculated. In a further analysis – stimulated by evidence that the morphology and histochemistry of the distal colon are related more closely to the rectum than the proximal colon[50-53,57] – we also combined those colon cancer cases having tumors diagnosed distal to the splenic flexure with the rectal cancer cases, and examined the effect of serum 25-OH D.

Statistical analyses

A paired t-test was used to test the hypothesis that the geometric means for the prediagnostic serum levels of 25-OH D and 1,25-DIOH D were the same for the cases and controls. Since two controls were matched per case, values for each control set were averaged to allowed for matched case-control pair comparisons. Conditional logistic regression techniques[58] were used to examine the association between large bowel cancer risk and prediagnostic serum levels of the two metabolites. Quartiles of exposure for each metabolite were created using the distribution of serum metabolite levels among the controls. In order to conduct linear trend analyses, variables were created using exposure scores based on the median values of each metabolite for the first to fourth quartiles among the controls. Modification of the effect of one metabolite by the other on large bowel cancer risk was examined by creation of scatter plots and statistical tests of the first-order interaction term in the conditional logistic regression models. Potential confounding of the associations between the serum metabolites and cancer risk by other related risk factors for large bowel cancer was explored using correlation analysis and multivariate regression modeling. Potential confounding variables were evaluated in stepwise fashion in conditional logistic regression models containing the serum vitamin D variables (as both indicator variables and trend variables). If the potential confounder caused a significant change in the model log likelihood statistic ($P \leq 0.05$) and a greater than 20 percent change in the serum vitamin-D-metabolite beta coefficient, it was kept in the model for further multivariate analysis. Neither exclusion of early cases nor multivariate adjustment for potential confounders materially altered risk estimates for either serum metabolite.

Therefore, univariate estimates of association (controlled only for matching factors) are presented throughout this report. All analyses were conducted using SAS statistical software.[59]

Results

Cases and controls were matched closely on age, month of blood draw, and study clinic. The median age of cases was 59 years compared with 60 years for controls. Almost 40 percent of cases and controls had their blood drawn during the spring months compared with only seven percent during the summer months.

A comparison of available data on anthropometric, dietary, lifestyle, and serum variables that could be related to colorectal cancer risk yielded a few differences between cases and controls (Table 1). Cases were heavier, had a larger BMI and had a significantly higher energy intake than controls, but physical activity did not differ by case-control status. Baseline serum total cholesterol was significantly lower in cases than controls. There was remarkable similarity between cases and controls in the distribution of other available baseline variables possibly related to colorectal cancer risk (Table 1). Baseline measures of serum α-tocopherol did not differ by case-control status. Although control subjects had higher levels of baseline serum β-carotene than cases, the difference was not statistically significant. Cases and controls also did not differ in a number of variables related to lifestyle – including level of education, marital status, and place of residence (urban/rural). Since both cases and controls were participants in a clinical trial for lung cancer prevention that required smoking as an entry criterion, similarity between the two groups in the number of cigarettes smoked per day and the number of years smoked was not unexpected. Among the dietary variables, measures of intake of the macronutrients fat and fiber did not differ between the two groups, nor did dietary intake of calcium or vitamin D. Only four percent of the study population took vitamin D in supplement form and there was no difference between cases and controls in frequency or amount of supplemental vitamin D.

Table 1. Selected baseline characteristics of colorectal cases and controls,[a] Finnish men

Characteristic	Cases (*n* = 146)		Controls (*n* = 292)	
Anthropometric				
Height (cm)	173.5	(5.9)	173.3	(6.3)
Weight (kg)	80.6	(11.7)	78.3	(12.2)
Body mass index (wt/ht^2)	26.8	(3.8)	26.0	(3.5)
Serum				
Total cholesterol (mmol/l)	6.0	(1.1)	6.3	(1.2)
α-tocopherol (mg/l)	11.8	(3.7)	12.0	(3.2)
β-carotene (ug/l)	184.0	(124.1)	200.0	(122.7)
Lifestyle				
Years smoked regularly	37.6	(8.8)	38.1	(8.3)
Cigarettes/day	20	(9)	19	(9)
Physical exercise > 3 times per week	26%		21%	
≤ Common school education[b]	77%		79%	
Married	75%		82%	
Urban residence	69%		67%	
Diet (daily intake)				
Energy (kcal)	2,812.1	(725.6)	2,625.8	(658.1)
Fat (g/1,000 kcal)	43.6	(5.9)	43.4	(6.6)
Fiber (g/1,000 kcal)	9.2	(2.8)	9.1	(3.0)
Protein (g/1,000 kcal)	36.4	(5.8)	37.0	(4.9)
Calcium (g)	1.3	(0.6)	1.3	(0.5)
Vitamin D (ug)	5.6	(3.0)	5.1	(2.9)

[a] Based on unmatched data with continuous variables expressed as the mean (standard deviation).
[b] Equivalent to six years to eight years.

Prediagnostic serum levels of the two vitamin D metabolites, 25-OH D and 1,25-DIOH D, for colorectal cases and controls are shown in Table 2. Although baseline geometric mean values for serum levels of the active vitamin D metabolite, 1,25-DIOH D, did not differ by case status, serum levels for 25-OH D were significantly lower for cases compared with controls (Table 2).

No consistent patterns in risk were observed for serum 1,25-DIOH D for either colorectal cancer combined or colon and rectal cancer separately (Table 3). A modest trend in reduced risk with increasing baseline serum levels of 25-OH D was observed for colorectal cancer, with over a 40 percent reduction in risk for the fourth quartile compared with the first quartile (Table 4). This trend was less evident for colon cancer and seemed to be driven by a stronger protective association seen for rectal cancer. Those in the upper quartile of serum 25-OH D exhibited only about one-third the risk of rectal cancer compared with those in the first quartile. Excluding cases who were diagnosed within the first two years of blood collection had no effect on the risk estimates for colon cancer and did not attenuate the protective association observed for rectal cancer (Table 4). In fact, the monotonic dose-response relation between serum levels of 25-OH D and rectal cancer risk was still evident and statistically significant. Adjustment for treatment group, and baseline covariates which were correlated with either of the serum vitamin D metabolites (*e.g.*, serum α-tocopherol, dietary vitamin E, folate, and vitamin C) and were possible risk factors for colorectal cancer did not materially affect risk estimates for colon and/or rectal cancer for either 25-OH D or 1,25-DIOH D. Assessment of the joint effect

Table 2. Prediagnostic serum levels of vitamin D metabolites by case-control status, Finnish men

	Cases (*n* = 146)	Controls (*n* = 290)	
25-hydroxyvitamin D (ug/l)			
Geometric mean (CI)[a]	12.2 (11.2-13.2)	13.8 (13.1-14.5)	*P* value[b] < 0.01
Interquartile range	8.7-17.7	9.8-19.1	
1,25-dihydroxyvitamin D (ng/l)			
Geometric mean (CI)[a]	36.5 (35.2-37.8)	36.7 (35.7-37.7)	*P* value > 0.50
Interquartile range	32.0-41.8	31.8-43.0	

[a] CI = 95% confidence interval.
[b] Paired *t*-test.

Table 3. Risk of adenocarcinoma of the large bowel by serum level of 1,25-dihydroxyvitamin D, Finnish men

	Quartiles[a] (ng/l)	Cases	Controls	RR[b]	(CI)[c]
Large bowel cancer (All colon + rectum)	Q1: ≤ 31.7	32	72	1.0	—
	Q2: >31.7 ≤ 37.3	43	72	1.3	(0.8-2.4)
	Q3: > 37.3 ≤ 43.1	41	74	1.2	(0.7-2.2)
	Q4: > 43.1	30	72	0.9	(0.5-1.7)
	P trend			0.76	
Colon cancer					
	Q1: ≤ 31.7	20	51	1.0	—
	Q2: > 31.7 ≤ 37.3	29	44	1.7	(0.8-3.4)
	Q3: > 37.3 ≤ 43.1	20	41	1.2	(0.6-2.6)
	Q4: > 43.1	22	45	1.2	(0.6-2.6)
	P trend			0.80	
Rectal cancer					
	Q1: ≤ 31.7	12	21	1.0	—
	Q2: > 31.7 ≤ 37.3	14	28	0.7	(0.3-2.2)
	Q3: > 37.3 ≤ 43.1	21	33	1.0	(0.4-2.5)
	Q4: > 43.1	8	27	0.5	(0.2-1.5)
	P trend			0.39	

[a] Quartile cutoffs based on the distribution in the control population.
[b] Univariate estimates of relative risk (RR) from conditional logistic regression.
[c] CI = 95% confidence interval.

of both serum vitamin D metabolites on colon and/or rectal cancer risk produced no significant interactions. Visual inspection of the scatterplots of serum levels of 25-OH D and 1,25-DIOH D for cases and controls also yielded no obvious patterns.

In the anatomic subsite analyses, the estimated relative risk (RR) for proximal colon cancer for the highest quartile of serum 25-OH D compared with the lowest quartile was 1.3 (95 percent confidence interval [CI] = 0.4-4.2) while, for distal colon cancer, the estimated RR was 0.6 (CI = 0.2-1.5), suggesting a protective association between 25-OH D and distal but not proximal colon cancer (Table 5). When we combined distal colon cases with rectal cancer cases, we observed a significant monotonic protective dose-response for serum 25-OH D. When comparing the highest quartile with the lowest quartile, we observed more than a twofold reduction in risk (Table 5). Once again, this association was not modified by 1,25-DIOH D levels and was not confounded by baseline covariates evaluated in earlier analyses.

Discussion

Our finding of an apparent protective association of vitamin D on large bowel cancer (especially rectal) risk is consistent with some but not all of the available analytic epidemiologic data. For example, two large prospective-cohort studies[7,8] found an inverse association between

Table 4. Risk of adenocarcinoma of the large bowel by serum level of 25-hydroxyvitamin D, Finnish men

	Quartiles[a] (ng/l)	Cases	Controls	RR[b]	(CI)[c]
Large bowel cancer (All colon + rectum)	Q1: ≤ 9.8	46	72	1.0	—
	Q2: > 9.8 ≤ 13.9	35	73	0.7	(0.4-1.3)
	Q3: > 13.9 ≤ 19.3	36	73	0.8	(0.4-1.3)
	Q4: > 19.3	29	72	0.6	(0.3-1.1)
	P trend			0.13	
Colon cancer					
	Q1: ≤ 9.8	30	47	1.0	—
	Q2: > 9.8 ≤ 13.9	18	47	0.6	(0.3-1.2)
	Q3: > 13.9 ≤ 19.3	22	45	0.8	(0.4-1.6)
	Q4: > 19.3	21	42	0.8	(0.4-1.6)
	P trend			0.69	
Colon cancer[d]					
	Q1: ≤ 9.8	19	38	1.0	—
	Q2: > 9.8 ≤ 13.8	13	35	0.8	(0.3-1.8)
	Q3: > 13.8 ≤ 19.2	19	32	1.2	(0.5-2.8)
	Q4: > 19.2	17	31	1.2	(0.5-2.8)
	P trend			0.58	
Rectal cancer					
	Q1: ≤ 9.8	16	25	1.0	—
	Q2: > 9.8 ≤ 13.8	17	26	0.9	(0.4-2.4)
	Q3: > 13.8 ≤ 19.2	14	28	0.8	(0.3-2.0)
	Q4: > 19.2	8	30	0.4	(0.1-1.1)
	P trend			0.06	
Rectal cancer[d]					
	Q1: ≤ 9.8	12	15	1.0	—
	Q2: > 9.8 ≤ 13.8	13	16	0.8	(0.2-2.4)
	Q3: > 13.8 ≤ 19.2	7	21	0.4	(0.1-1.3)
	Q4: > 19.2	7	25	0.3	(0.1-1.1)
	P trend			0.04	

[a] Quartile cutoffs based on the distribution in the control population.
[b] Univariate estimates of relative risk (RR) from conditional logistic regression.
[c] CI = 95% confidence interval.
[d] Excluding cases diagnosed within 2 years of blood collection.

vitamin D intake from diet and/or supplements and large bowel cancer risk. In a study of 1,954 men conducted by Garland and colleagues,[7] the incidence of colorectal cancer among men in the highest quartile of vitamin D dietary intake was almost half the incidence observed in the first quartile. Bostick *et al*[8] studied a cohort of over 35,000 women and observed an inverse association between total intake (diet and supplements) of vitamin D and colon cancer risk. The age-adjusted RR for the highest *cf* lowest quintile was 0.5 (CI = 0.3-0.8) but was attenuated to 0.7 (CI = 0.4-1.2) upon multivariate adjustment.[8] The prospective nature of these findings along with the strength of the observed inverse association between vitamin D intake and risk of large bowel cancer make these studies interesting. They are limited, however, in that they did not measure the main contributor to overall vitamin D status, *i.e.*, sun exposure, nor did they measure a more direct indicator of vitamin D status such as the serum level of the vitamin D metabolite, 25-OH D.

Three studies[9,10,60] that did examine the serum levels of vitamin D metabolites and colon cancer risk produced different results. Interestingly, both studies used a nested case-control design and were conducted in the same population (using different cases and controls) approximately six years apart. In the earlier study, Garland *et al*[9] found that colon cancer cases (n = 34) had significantly lower serum levels of 25-OH D than their matched controls (n = 67), and that low serum levels of 25-OH D were associated with increased colon cancer risk. However, there was no evidence of a protective trend when they examined the relative odds of colon cancer by increasing quintile of serum 25-hydroxyvitamin D. Serum 1,25-

Table 4. Risk of adenocarcinoma for different locations in the large bowel by serum level of 25-hydroxyvitamin D, Finnish men

	Quartiles[a] (ng/l)	Cases	Controls	RR[b]	(CI)[c]
Proximal colon cancer[d]					
	Q1: ≤ 9.8	13	26	1.0	—
	Q2: > 9.8 ≤ 13.9	6	22	0.5	(0.2-1.6)
	Q3: > 13.9 ≤ 19.3	13	19	1.4	(0.5-4.1)
	Q4: > 19.3	11	19	1.3	(0.4-4.2)
	P trend			0.44	
Distal colon cancer[e]					
	Q1: ≤ 9.8	21	26	1.0	—
	Q2: > 9.8 ≤ 13.9	15	22	0.7	(0.3-1.7)
	Q3: > 13.9 ≤ 19.3	2	21	0.4	(0.2-1.2)
	Q4: > 19.3	10	26	0.6	(0.2-1.5)
	P trend			0.25	
Distal colon and rectal cancer[f]					
	Q1: ≤ 9.8	33	47	1.0	—
	Q2: > 9.8 ≤ 13.8	29	50	0.8	(0.4-1.6)
	Q3: > 13.8 ≤ 19.2	23	54	0.6	(0.3-1.2)
	Q4: > 19.2	18	53	0.5	(0.2-0.9)
	P trend			0.03	

[a] Quartile cutoffs based on the distribution in the control population.
[b] Univariate estimates of relative risk (RR) from conditional logistic regression.
[c] CI = 95% confidence interval.
[d] Proximal colon cancer includes tumors in the cecum (16), appendix (1), ascending colon (10), transverse colon (6) and hepatic flexure (10).
[e] Distal colon cancer includes tumors in the splenic flexure (3), descending colon (4), unspecified colon (2), and sigmoid colon (39).
[f] Rectal cancer includes tumors in the rectosigmoid junction (5), rectum (49) and anal canal (1).

DIOH D was not measured in Garland's study. More recently, in this same population but using different cases (n = 57) and matched controls (n = 114), Braun *et al* [10,60] found no significant differences in mean serum levels of either 25-OH D or 1,25-DIOH D, nor did they find a protective association between either metabolite and colon cancer incidence. This study differed from that of Garland *et al* in that the interval between the date of blood collection and colon cancer diagnosis was longer (< 1-8 ys [Garland] *cf* 10-17 yrs [Braun]). However, Braun found no evidence to suggest that the differences between case and control serum 25-OH D could be explained by the interval between collection and diagnosis. Both studies were limited to colon cancer alone and, therefore, could not examine the relation between rectal cancer risk and serum levels of vitamin D metabolites.

Although our colorectal cases had significantly lower serum levels of 25-OH D than their matched controls and we did observe a modest dose-response trend with increasing baseline serum 25-OH D, our findings differed when we considered colon and rectal cancer separately. Our failure to observe a significant relation between serum 25-OH D and colon cancer risk was concordant with the findings of Braun *et al.* [10,60] Although our RR point estimates for quartiles 2 through 4 compared with quartile 1 were all less than one, none of the CIs excluded unity and a dose-response effect was not evident. Our findings for the hormonal form of vitamin D, 1,25-OH D, and colon cancer risk were also concordant with Braun *et al.* In fact, we found no consistent patterns of association between 1,25-DIOH D and colorectal, colon, or rectal cancer risk.

We did observe an apparent protective association for serum 25-OH D and rectal cancer risk. Those in the highest quartile of baseline serum 25-OH D were more than 2.5 times less likely to develop rectal cancer compared with those in the lowest quartile. This association persisted even after excluding early cases (occurring within two years of baseline blood collection) and adjusting for a number of potential confounding variables. These findings raise two important questions: Why did we observe a protective association for serum levels of 25-OH D and not the active hormonal form of the vitamin, 1,25-DIOH D?; and, why did we observe an apparent protective association for 25-OH D and rectal cancer risk but not colon cancer risk?

The principal circulating metabolite of vitamin D, 25-OH D, serves as the requisite precursor of the active hormonal form of the vitamin, 1,25-DIOH D. The serum level of 25-OH D is not maintained homeostatically and is established as a clinically useful measurement of overall vitamin D status whether attained through photobiosynthesis of vitamin D in the skin or dietary intake of vitamin D. This metabolite, although subject to seasonal variation, has a relatively long half-life (three to four weeks) and exhibits little intra-individual diurnal variation (within a given season).[61] Although 1,25-DIOH D is accepted as the active hormonal form of the vitamin, its measurement provides essentially no information regarding a person's nutritional vitamin D status. Its relatively short half-life (four to six hours) in the circulation and its tight homeostatic control based on a number of factors related to calcium and phosphate balance may limit its clinical utility.[62] Although the affinity of 25-OH D for the vitamin D receptor is more than an order of magnitude less than 1,25-DIOH D, serum levels of 25-OH D are typically three orders of magnitude greater than 1,25-DIOH D. Further, there is a modest amount of evidence that 25-OH D may have a direct physiological role other than as a precursor for 1,25-DIOH D.[63-66]

With regard to vitamin D and cancer, there is also limited experimental and epidemiologic evidence suggesting that overall vitamin D status, as measured by serum levels of 25-OH D, can modify the effect of 1,25-DIOH D on carcinogenesis.[67,68] At least one *in vitro* study[69] provides evidence to suggest that, in certain cancer cells from solid tumors, constitutive synthesis of the active hormone (1,25-DIOH D) may take place within tissue at the site of neoplasia through the action of the cytochrome P450 dependent enzyme, 1 α-hydroxylase, on 25-OH D. This effect is substrate (25-OH D) dependent and is not stimulated by parathyroid hormone.[69]

The apparent protective association of higher serum levels of 25-OH D observed for rectal cancer but not colon cancer may have been a chance finding or may have some basis in large bowel morphology and physiology. Some argue that, especially when examining neoplastic processes, the large bowel should be looked upon not by the classic anatomic distinction of colon and rectum, but rather as segments proximal and distal to the splenic flexure. There is evidence to suggest that these segments may have different susceptibilities to neoplasia given their different embryologic origins, different expression of cell membrane glycoproteins, different patterns of cell-surface antigens, and different morphologic and histochemical characteristics. Bufil[50] cites both epidemiologic and cytogenetic evidence to support his theory that there are distinct genetic categories of large bowel cancer by proximal and distal tumor location. For example, there are data to indicate that tumor DNA content, oncogene expression and allelic deletions differ by tumor location in the bowel and that this may suggest distinct pathogenetic and biologic behavior at proximal and distal sites. In contrast to tumors in the proximal colon, distal tumors often have hyperploid DNA content, frequent allelic deletions, an unstable karyotype, and deregulation of *c-myc* expression is common.[50]

Interestingly, one of the mechanisms proposed for vitamin D's action in cell proliferation and differentiation involves control of expression of the *c-myc* oncogene.[70-73] Although the exact mechanisms by which vitamin D exerts its effects on cell proliferation and differentiation are unknown, there is evidence to support both genomic (including control of oncogene expression) and non-genomic activity.[74,75] If deregulation of *c-myc* expression is important in the pathogenesis of distal large bowel tumors and the expression of *c-myc* oncogene is somehow regulated by vitamin D, this may provide at least a partial explanation for the different results we observed for the proximal and distal large bowel.

The presence of vitamin D receptors in normal mucosa throughout the whole length of the large bowel also provides evidence that vitamin D has a physiologic role in this organ.[76-78] In one study, the relative abundance of vitamin D receptors in the large bowel was lower in adenocarcinoma compared with normal mucosa, and the number of receptors in tumors decreased from proximal to distal location.[77] The authors found no correlation between presence of receptor in adenocarcinomas with the age and gender of the patient or the degree of extension of the tumor (Dukes' stage). It is possible that the loss of receptors in tumor tissue is related to an alteration of the gene expression associated with the neoplastic process. The fact that this receptor loss differed in tumors in the proximal and distal large bowel argues for potential differences in pathophysiology by location and possibly different susceptibilities to a vitamin D effect.

One of the strengths of this study is its prospective design. The fact that the collection of questionnaire covariate data and blood specimens took place well before case diagnosis minimizes the potential for recall bias and a potential disease effect on serum measurements. Although it is possible that undetected preclinical cancers may have affected levels of the serum vitamin D metabolites, results of our statistical analyses of rectal cancer which excluded cases diagnosed within two years of blood collection did not support this hypothesis. Our observation of a relatively strong negative association between serum levels of 25-OH D and rectal cancer risk makes it less likely this association was spurious. This finding was strengthened further by the presence of a significant monotonic dose-response relation across the gradient of serum 25-OH D.

Our ability to detect an association between serum

25-OH D and cancer risk may have been enhanced by the overall low-vitamin-D status of our population. Given its northern latitude with long periods of little or no sunlight and lack of vitamin D food fortification, one would expect the Finland population to have lower levels of the main circulating metabolite of vitamin D, 25-OH D. In fact, we observed that one-quarter of our study population could be classified at or below the borderline of vitamin D deficiency (< 10 ng/ml). It is possible that our observation of a significant association between serum 25-OH D and rectal cancer risk may have been due to our ability to tease out group differences better at the very low end of the dose-response curve (represented by our population) than is possible in populations with few or no deficient persons.

In summary, we observed a downward trend of risk of colorectal cancer with increasing serum levels of 25-OH D but not with 1,25-DIOH D. This relation was stronger in rectal cancer than colon cancer with more than a two-fold reduction in risk in the highest quartile of baseline serum 25-OH D compared with the lowest quartile. This association persisted, and indeed, even was strengthened by the exclusion of cases diagnosed within two years of baseline blood collection, diminishing the probability that the association observed was due to preclinical rectal cancer cases. Although the generalizability of these findings is somewhat limited by the select nature of the study subjects (male smokers participating in a prevention trial), this study supports previous reports that adequately high circulating levels of vitamin D are associated with reduced risk of colorectal cancer. This connection between vitamin D status and large bowel cancer risk warrants further examination in other populations.

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