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# Epidemiology of Vitamin D and Colorectal Cancer

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**Abstract:** Garland and Garland first hypothesized that better vitamin D status lowered risk of colorectal cancer in 1980. Subsequently, the relation between vitamin D status and colorectal cancer risk has been investigated in epidemiologic studies. Various approaches have been used to estimate vitamin D status, including direct measures of circulating 25(OH)vitamin D levels, surrogates or determinants of vitamin D (including region of residence, intake, and sun exposure estimates, or a combination of these). These measures of vitamin D status have been studied in relation to colorectal adenoma, precursors of cancer, and colorectal cancer incidence and mortality.

In general, all lines of inquiry from observational studies indicate that better vitamin D status is associated with lower colorectal cancer risk. While most of the studies have examined vitamin D status in relation to risk of incident colorectal cancer, some evidence suggests that vitamin D may be additionally important for colorectal cancer progression and mortality. Although the influence of confounding factors cannot be entirely excluded, the consistency of the association using various approaches to measure vitamin D, for diverse endpoints and in diverse populations shows high consistency and is strongly suggestive of a causal association. Thus, improving vitamin D status could be potentially beneficial against colorectal cancer incidence and mortality.

**Keywords:** Vitamin D, Cholecalciferol, Colorectal cancer, Colorectal adenoma, Epidemiology, Ultraviolet B.

## INTRODUCTION

Colorectal cancer is the third most common type of cancer in both men and women worldwide. Globally, over 1.2 million new cases are diagnosed and approximately 600,000 deaths are attributed to this malignancy annually. In the Unites States, the American Cancer Society estimates 103,000 new cases of colon cancer and 40,000 new cases of rectal cancer in 2012 [1]. The lifetime risk for an individual in the United States is approximately 5 per cent. The estimated number of deaths in 2012 in the United States is 51,690. Incidence and mortality rates are even higher in some European countries, and Japan has among the highest rates. Colorectal cancer exhibits large variations in incidence and mortality rates across countries and regions, with rates generally higher in economically developed countries. While genetic factors are important, much of the variation in rates appears attributable to modifiable factors, including lifestyle and diet.

In 1980, Garland and Garland noticed that colon cancer mortality rates in the United States varied by region, with rates generally being higher in the northern regions that receive relatively low solar ultraviolet B (UV-B) radiation. Because UV-B is required to synthesize vitamin D in the skin, these investigators then hypothesized that poor vitamin D status accounted for the higher mortality rates of colon cancer [2]. This publication marked the beginning of the vitamin D - cancer hypothesis. Subsequent to this publication, this topic became of high interest, with numerous basic science and epidemiologic studies addressing whether there may be a causal association between vitamin D status and risk of colorectal cancer.

Although assessing the likelihood of a causal association ultimately relies from human studies, the biologic plausibility is an important component in addressing whether the evidence supports a causal association. In the past several decades, laboratory studies have uncovered various anti-carcinogenic properties of vitamin D. In the 1970's and 1980's, many discoveries indicated that the role of vitamin D extended beyond the traditional effects on calcium and phosphorus homeostasis [3]. Cells in various tissues were discovered to have the capacity to convert 25(OH)vitamin D (25(OH)D) to 1,25 dihydroxyvitamin D ($1,25(OH)_2D$), the active form that activates the vitamin D receptor (VDR) to influence a variety of transcriptional changes. Levels of 25(OH)D that are inadequate to maintain physiologic concentrations of $1,25(OH)_2D$ in cells cause aberrations in pathways including differentiation, proliferation, invasiveness, angiogenesis, and metastatic potential. Thus, individuals or populations with inadequate or deficient 25(OH)D levels, may in the course of time, be pre-disposed to a higher risk of cancer and possibly more advanced or aggressive malignancies. Various animal models indicate that vitamin D status influences growth of intestinal tumors [4-7], and in human colorectal cancer cell lines, 1,25(OH)D induces apoptosis in a dose-dependent manner [8].

From human studies, important criteria to be considered for causality are the consistency of evidence, the strength of the association, and the temporality of the relationship. If an association is observed, the consideration of confounding factors is important. A confounder is a factor that is associated with vitamin D status and is causally associated with colorectal cancer that causes a spurious association (that is, non-causal) between vitamin D status and colorectal cancer risk. The relationship between vitamin D status and colorectal cancer has been studied using a variety of surrogates of vitamin D status, including estimates of solar radiation at the population (ecologic) level or individual level, studies based on circulating 25(OH)vitamin D levels (or predicted 25(OH)D levels), and studies based on dietary and supplementary intakes. The evidence from randomized trials has been limited. Endpoints considered in these studies have been colorectal adenoma incidence, colorectal cancer and mortality, and survival in patients with colorectal cancer.

## GEOGRAPHIC STUDIES AS A SURROGATE OF UV-B EXPOSURE

In ecologic studies, vitamin D status is inferred – for example, residence in regions with greater annual solar UV-B exposure may allow for greater opportunity for vitamin D synthesis from sun exposure, but actual exposure depends on the individuals' behaviors. One potentially important advantage of these studies is that most serum-based and dietary cohorts are composed of middle-aged individuals, and the assessment of past sun exposures allows for estimating vitamin D status at points earlier in life, at least based

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on region of residence. For cancer risk, these earlier time periods could be most relevant because the time relation between vitamin D and cancer risk is not known. Ecologic studies are also feasibly done in many settings. The main limitation for most ecologic studies may be the difficulty in controlling for various potential confounding factors. Another important limitation is that this approach does not directly assess vitamin D status, and region is not a perfect surrogate of vitamin D status.

Ever since the relationship between regional solar UV-B and lower risk of colon cancer mortality was proposed by Garland and Garland [2], this finding has been confirmed in the United States and in other settings. Grant showed that regional UV-B radiation correlated inversely with mortality rates of multiple cancers, and observed the strongest association with colorectal cancer mortality [9]. Another analysis of United States data that examined both incidence and mortality showed inverse associations for both, but more strongly for colorectal cancer mortality than for incidence [10]. In that study, relative to regions with higher UV-B exposure, the relative risk (RR) for incidence in regions with lower UV-B was 1.18 in men and 1.14 in women for colon cancer, and 1.27 in men and 1.14 in women for rectal cancer. For mortality, these respective relative risks were even higher: 1.27 (M) and 1.24 (F) for colon cancer and 1.53 (M) and 1.37 (F) for rectal cancer. These relative risks were based on national data over a number of years and the confidence intervals were thus very tight. Adjustment based on various population data sources were made for age, poverty, income, smoking, alcohol, exercise, outdoor occupation, rural/urban, and air quality, but the association with region persisted.

Importantly, the association between regional UV-B exposure and lower colorectal cancer risk has been observed in different populations outside of the United States. For example, in Japan, Mizoue calculated Pearson correlation coefficients between averaged annual solar radiation levels for the period from 1961 through 1990 and cancer mortality in the year 2000 in 47 Japanese prefectures [11]. After adjusting for regional per capita income and dietary factors, an inverse correlation was shown between averaged annual solar radiation levels and mortality from colon cancer in men (r= –0.53) and in women (r=–0.46), and for rectal cancer (r= –0.53 in men, and r =–0.47 in women). In a study from China, regional cancer mortality rate was associated inversely with UV-B but cancer incidence rate was not, also suggesting a stronger role of UV-B on mortality rates than incidence rates [12].

Regional solar UV-B was studied in an individual-based case-control study design (opposed to population-based or ecologic study) of cancer mortality by use of death certificates in the United States [13]. This study examined residential and occupational exposure to sunlight in relation to mortality from colon cancer; non-melanoma skin cancer served as a positive "control" because high sun exposure would be expected to increase risk of this cancer. In this very large study, the cases consisted of all colon cancer deaths between 1984 and 1995 in 24 states, and the controls were age-frequency matched, with deaths from cancer and certain neurological diseases excluded from controls because of possible relationships with sun exposure. The multivariate analyses controlled for age, sex, race, and mutual adjustment for residence, occupation (outdoor versus indoor), occupational physical activity levels and socioeconomic status. Based on 153,511 cases of colon cancer deaths, those with high compared to low exposure to sun based on residence were at decreased risk (relative risk (RR) = 0.73; 95% confidence interval (CI): 0.71–0.74). In addition, individuals who had had outdoor occupations (RR = 0.90; 95% CI: 0.86–0.94) and occupations that required more physical activity (RR = 0.89; 95% CI: 0.86–0.92) were at lower risk. The inverse association observed with outdoor occupation was strongest among those living in the highest sunlight region.

## NESTED CASE-CONTROL STUDIES OF 25(OH)D AND COLORECTAL CANCER OR ADENOMA RISK

Studies that have examined levels of circulating 25(OH)D concentration in relation to risk of colorectal cancer have been typically based on a cohort with archived blood samples. 25(OH)D is typically measured in ng/mL or nmol/L (1 ng/mL = 2.496 nmol/L). After a specified follow-up period, blood samples from cases who developed colorectal cancer and selected controls are retrieved and 25(OH)D is measured. Analyses then compare 25(OH)D levels in cases and controls to calculated the odds ratio, which is an estimate of the relative risk or RR. This study design is efficient and helps prevent bias in that the level of circulating marker is representative of the pre-diagnostic period, and is not influenced by disease status. However, it is important to examine results with different time lags between the blood draw and the diagnosis time period as undiagnosed latent cancers could theoretically affect blood levels of 25(OH)D, leading to biased results (reverse causation). A limitation is that a single blood draw and variable follow-up may imperfectly capture the time period that vitamin D is etiologically relevant.

Pooled analyses (meta-analyses) of studies on 25(OH)D levels confirm a significant inverse association [14-16]. In a 2011 meta-analysis, based on 1,822 colon and 868 rectal cancers, increasing circulating 25(OH)D level was associated with a significant reduction in colorectal cancer (top vs. bottom quantiles, OR = 0.66; 95% CI: 0.54–0.81) [15]. Controlling for multiple covariates had little influence on the findings. The results across studies are somewhat inconsistent in distinguishing whether the association is stronger for colon cancer or for rectal cancer, possibly due to small numbers, but in general the association has been observed for both anatomic sites. In the meta-analysis, the inverse association was stronger for rectal cancer (OR = 0.50; 95% CI: 0.28–0.88) than colon cancer (OR = 0.77; 95% CI: 0.56–1.07) but no significant difference was noted (p value for difference between colon and rectal cancer = 0.20) [15]. Another meta-analysis published in 2011 found that a 10 ng/mL increment in 25(OH)D was associated with a RR of 0.74 (95% CI: 0.63–0.89) [17]. There was no evidence of heterogeneity among the studies and no evidence of deviation from a linear inverse association, at least up to levels of approximately 40 ng/mL. There were few data points above this level. The results were very similar for studies conducted in the United States (RR = 0.61; 95% CI: 0.43–79), Europe (RR = 0.72; 95% CI: 0.51–0.92) and Asia (RR = 0.84; 95% CI: 0.35–1.32).

Individually, the four largest studies were from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, a combined analysis of the Nurses' Health Study and Health Professionals Follow-Up Study [18], the Women's Health Initiative (WHI), and the Japan Public Health Center-based Prospective Study. In the EPIC study, 1,248 colorectal cancer cases were identified and matched to 1248 control subjects by age, gender, study center, follow-up time, fasting status, and time of day of blood donation [19]. Compared to a serum 25(OH)D concentration of 20-29 ng/mL, lower levels of a 25(OH)D were associated with an increase in colorectal cancer risk (<10 ng/mL: OR = 1.32; 95% CI: 0.87–2.01; 10-19 ng/mL: OR = 1.28; 95% CI: 1.05–1.56), whereas higher concentrations were associated with a decreased risk of colorectal cancer (30-39 ng/mL: OR = 0.88; 95% CI: 0.68–1.13; ≥40 ng/mL: RR = 0.77; 95% CI: 0.56–1.06) [19]. The association was stronger in the colon versus the rectum.

In the combined results from the Health Professionals Follow-Up Study (HPFS) and the Nurses' Health Study (NHS), higher plasma 25(OH)D levels were associated with decreased risks of both colorectal cancer (RR = 0.66; 95% CI: 0.42–1.05; p(trend) = .01) and colon cancer (RR = 0.54; 95% CI: 0.34–0.86; p(trend) = .002) [18]. The results for rectal cancer were inconsistent between the two cohorts, though the number of cases was small. This study

adjusted for age, body mass index, physical activity, smoking, family history, use of hormone replacement therapy (in women only), aspirin use, and dietary intakes of various factors. The HPFS plasma-based study encompassed 179 cases. In a further analysis of the entire HPFS cohort, an estimate of circulating 25(OH)D (termed "predicted 25(OH)D") was used based on how various factors predicted 25(OH)D levels, and this score was examined in relation to subsequent risk of incident colorectal cancer cases, based on 691 incident cases. [20]. For the first step, in a sample of 1,095 men from the cohort, actual plasma 25(OH)D level was the dependent variable in a multiple linear regression. The independent variables were derived from questions that the entire cohort of 47,000 men had responded to, including geographical region, skin pigmentation, dietary intake, supplement intake, body mass index, and leisure-time physical activity, a surrogate of potential exposure to sunlight UV-B [21]. Then, based on the regression coefficients from this sub-study, a predictor score was calculated for each of 47,000 participants from the entire cohort. In the multivariable analysis, a 10 ng/mL increment in 25(OH)D was associated with a 37% reduced risk of colorectal cancer (RR = 0.63; 95% CI: 0.48–0.83). This inverse association persisted after controlling for body mass index and physical activity.

The Women's Health Initiative was based on a total of 322 cases of colorectal cancer [22]. In that study, an inverse association was observed between baseline 25(OH)D level and colorectal cancer risk; however, detailed analyses on potential confounders were not reported. Plasma 25(OH)D and colorectal cancer incidence risk was examined in The Japan Public Health Center-based Prospective Study [23], a nested case-control study of 375 incident cases of colorectal cancer during 11.5-years of follow-up after blood collection. Two controls were matched per case on sex, age, study area, date of blood draw, and fasting time. The multivariate analysis further adjusted for smoking, alcohol consumption, body mass index, physical exercise, vitamin supplement use, and family history of colorectal cancer. Plasma 25(OH)D was not significantly associated with colorectal cancer but the lowest category of plasma 25(OH)D was associated with an elevated risk of rectal cancer in both men (RR = 4.6; 95% CI: 1.0–20) and women (RR = 2.7; 95% CI: 0.94–7.6), compared with the other quartiles combined.

This association is further supported by a decrease in risk of colorectal adenomas, which are well established precursor lesions for colorectal cancer, with higher serum 25(OH)D. Most of the studies were based on comparing 25(OH)D levels in adenoma cases to controls who were adenoma-free on colonoscopy or sigmoidoscopy. The studies were based either on initial adenomas or on adenomas among individuals who had had an adenoma and were then followed for subsequent (recurrent) adenomas. A meta-analysis published in 2008 showed that 25(OH)D was inversely associated with risk of colorectal adenomas (high vs. low circulating levels, RR = 0.70; 95% CI: 0.56–0.87) [24]. The inverse associations appeared stronger for advanced adenomas (RR = 0.64; 95% CI: 0.45–0.90), but the number of studies was small [24]. Results have been similar in studies which have examined incident or recurrent adenomas. These results were confirmed in two more recent meta-analyses [25, 26]. The number of cases of adenomas now total to 3398.

## STUDIES BASED ON DIETARY AND SUPPLEMENTARY VITAMIN D INTAKE

In most populations, vitamin D is scare in (non-fortified) foods. Thus, vitamin D intakes are generally low compared to levels that would be required to minimize cancer risk. For example, a glass of fortified milk (in the United States) contains only 100 IU vitamin D, an amount that would increase 25(OH)D level by approximately one ng/mL. In populations with ample sun exposure, in contrast, "natural" levels of 25(OH)D can be 50 ng/mL or higher [27, 28]. For many individuals, intakes of several thousand IU of vitamin D per day are required to achieve a level of approximately 30 ng/mL. Although probably more vitamin D is made from sun exposure than is ingested in most populations, vitamin D intake is nonetheless a relatively important contributor to 25(OH)D levels, especially in winter months in regions at high latitudes, where vitamin D production is negligible. An important consideration of studies of vitamin D intake is that, depending on the specific population, intake of vitamin D may be predominantly from one or a few sources, such as fatty fish, fortified milk, or supplements, which could lead to confounding by these factors.

The association between colorectal cancer risk and dietary or supplementary vitamin D has been investigated in cohort studies of men [29, 30] and women [31-33] or both sexes [34, 35], and in case-control studies [36-43]. In a recent meta-analysis, based on prospective studies of vitamin D intake and colorectal cancer risk, with 9 identified studies, there was an inverse association for high versus low intakes (RR = 0.88; 95% CI: 0.80–0.96) [17]. No statistically significant heterogeneity was observed across studies, and the results were very similar for studies conducted in the United States (RR = 0.88), Europe (RR = 0.87) and Asia (RR = 0.87). The majority of dietary studies found inverse associations for colon or rectal cancer, or both [29-32, 35, 37, 39, 41, 42, 44]; studies that took into account supplementary vitamin D and where milk is fortified tended to get stronger results. In the studies with the highest intakes, the cut-point for the top category was from approximately 500 to 600 IU/day, and the average intake in this category was approximately 700-800 IU/day. This level of intake, expected to increase circulating 25(OH)D level by about 7-8 ng/mL, has been shown in randomized trials to reduce risk of fractures and falls [45, 46]; thus, it may biologically meaningful if not optimal.

Similar risk reductions between vitamin D intake and risk for colorectal adenoma have also been observed. A recent meta-analysis of 12 studies based on vitamin D intake [24] found that compared with the lowest quantile of vitamin D intake, the highest quantile was associated with an 11% marginally decreased risk of colorectal adenomas (odds ratio, 0.89; 95% CI: 0.78–1.02) and recurrent adenomas, (odds ratio = 0.88; 95% CI: 0.72–1.07), particularly for advanced adenoma (odds ratio = 0.77; 95% CI: 0.63–0.95).

Many of the studies described above controlled for known or suspected risk factors for colorectal cancer. Nonetheless, residual confounding is difficult to entirely exclude because the risk reductions are relatively modest. In particular, the independent effects of vitamin D and calcium intakes might be difficult to separate in populations that fortify milk with vitamin D. In such populations, milk may be the main source of both calcium and vitamin D intakes, which may make them highly correlated. Thus, some of the apparent benefit of vitamin D intake may be due to calcium intake, although some evidence shows that vitamin D intake remains associated with lower risk of colorectal neoplasia even after statistical adjustment for calcium intake [47]. Further, some evidence indicates that vitamin D and calcium may interact biologically in regards to colorectal cancer [48]. Supporting this, in a randomized trial of calcium intake and risk of adenoma, the level of 25(OH)D was associated with a reduced risk of adenoma only among subjects who were randomized to receive calcium [49].

Overall, the dietary intake studies of vitamin D are compatible with a lower risk of colorectal cancer and adenoma. The magnitude of the risk reduction associated with higher intakes is approximately 10-15%. This magnitude is approximately what would be expected based on the associations from the plasma and serum-based studies. The association is also relatively consistent in diverse populations. However, residual confounding is difficult to exclude, given the modest magnitudes of the associations.

## RANDOMIZED CONTROLLED TRIALS

The "gold standard" study in establishing a causal association is a double-blinded, placebo-controlled, randomized intervention trial because through randomization, confounding by other causal factors can be largely eliminated and various biases can also be avoided through blinding. Thus, in principle, the randomized intervention can provide the strongest evidence of a cause and effect association. However, in practice, randomized studies have a number of limitations in the study of nutrients and cancer. Because specific cancers, even relatively common ones such as colorectal cancers have a low annual incidence, studies must be large, typically involving tens of thousands of subjects, which renders them expensive to conduct. In addition, cancers have a long natural history and various factors could act preferentially at different stages. For example, the influence of ionizing radiation in increasing breast cancer typically is not seen for several decades after the exposure. Thus, randomized trials for cancer are heavily skewed towards the effects of promoters and may entirely miss effects of initiation or early effects. In regards to a nutritional factor, such as 25(OH)D level, unlike a pharmaceutical agent, for which the control group is truly unexposed, everyone has an underlying baseline level of 25(OH)D. Thus, the potential benefit from a vitamin D supplement would very likely vary among various populations, depending on the underlying vitamin D status. For example, in a population with adequate vitamin D status, an effect of additional vitamin D may not be observed. Additionally, randomized interventions may suffer from poor compliance, and contamination by the placebo group adopting the change (for example, taking vitamin D supplements outside of the study protocol). Historically, it is very likely that such limitations have contributed to null results in the study of nutritional factors in relation to risk of various cancers [50].

Currently, there are two randomized trials of vitamin D with meaningful numbers of cases of colorectal cancers. The largest such study is the Women's Health Initiative (WHI), which was a randomized placebo-controlled trial of 400 IU vitamin D plus 1000 mg/day of elemental calcium in 36,282 post-menopausal women [22]. Over a seven year period, the incidence of invasive colorectal cancer did not differ between women assigned to calcium plus vitamin D supplementation and those assigned to placebo (168 and 154 cases; hazard ratio, RR = 1.08; CI: 0.86-1.34; P = 0.51).

This study had some important limitations. First, the vitamin D dose of 400 IU/day was unlikely to generate a substantial contrast between the treated and the placebo groups as the expected increase of serum 25(OH)D would be about 3-4 ng/ml, assuming full compliance. In fact, adherence was sub-optimal and a high percentage of women took non-study supplements, so the actual contrast of 25(OH)D tested between the treated and the placebo group was likely further reduced. In the observational studies of 25(OH)D and colorectal neoplasia risk, the contrast between the high and low quintiles or quartiles of 25(OH)D was generally at least 20 ng/mL. Thus, the effect on risk associated with a small increment in 25(OH)D is likely to be barely perceptible, even assuming that the association in the observational studies is causal. Interestingly, in a re-analysis of the WHI limited-access dataset, a suggestive protective association of treatment was observed in women with no personal use of calcium and vitamin D (RR = 0.83; 95% CI: 0.60–1.15) but not in those with use of calcium or vitamin D supplements (RR = 1.26; 95% CI: 0.94–1.69; p(interaction) = 0.044) [51]. Another limitation is that the seven year duration for the trial may not have been sufficiently long to show an effect. The expected induction period for a vitamin D effect is unclear; one observational study suggested that at least ten years may be required to observe an effect of vitamin D intake on colorectal cancer risk [32].

An additional complexity of the WHI was that it had a factorial design along with hormonal replacement use (HRT), which has been shown to reduce risk of colorectal cancer. A post-hoc analysis suggested that women on hormones did not benefit from vitamin D and calcium, but subjects not taking hormones may have benefited (P (interaction) = 0.018 [52]. This finding suggests that additional vitamin D may not further lower risk of colorectal neoplasia among women at already reduced risk due to HRT use. Although this could have been a chance finding, some observational studies have subsequently supported this interaction. For example, in the Nurses' Health Study, no significant association and colorectal adenoma risk with vitamin D intake was observed for pre-menopausal women or for current users of post-menopausal hormones (HRT), but higher vitamin D intake was associated with lower adenoma risk among past users of HRT (RR = 0.56; 95% CI: 0.36–0.89; p trend = 0.03) and suggestively so but not significant among never users (RR = 0.82; 95% CI: 0.54–1.27; p trend = 0.37) [47]. In addition, one study found no association between outdoor time or ambient UV-B measure and colorectal cancer risk in current HRT users, but an inverse association with higher ambient UV exposure was found in never/past HRT users (RR for highest vs. lowest tertile = 0.40; 95% CI: 0.17–0.93; p for trend = 0.04) [53]. These findings need confirmation. A study of global gene expression in rectal mucosal biopsies suggested that the preventive action of HRT on colon neoplasia results, at least in part, from changes in vitamin D activity [54].

The second randomized controlled study that addressed vitamin D intake in relation to colorectal cancer risk was a study in the United Kingdom of 2686 subjects 65-85 years old. The subjects received either 100,000 IU of vitamin D every 4 months or placebo over a period of 5 years [55]. This amount of vitamin D is equivalent to 820 IU of vitamin D daily. After treatment, the 25(OH)D level was 29.7 ng/mL in the vitamin D treated group and 21.4 ng/mL in the placebo group, corresponding to about an 8 ng/mL increment in 25(OH)D due to supplementation. Over the five year period, 53 cases of colorectal cancer were documented; no association was associated with treatment relative to placebo (RR = 1.02; 95% CI: 0.60–1.74). This study was limited by the relatively small sample size, short follow-up and moderate dose of vitamin D.

## VITAMIN D AND SURVIVAL FROM COLORECTAL CANCER

Beyond influencing the risk of developing colorectal cancer (cancer incidence), some mechanisms suggest plausible effects of vitamin D on tumor aspects of aggressive behavior, and thus on cancer progression. Late stage anti-cancer effects of vitamin D, such as reduction in metastases, are observed in numerous animal models. Thus, potentially relevant endpoints in epidemiologic studies may be advanced stage or high grade cancer, metastasis, poorer survival and increased mortality. Some evidence suggests that any association of solar radiation or vitamin D on colorectal cancer risk may be stronger for cancer progression or for survival from cancer. As discussed previously, the geographical association between regional UV-B and colorectal cancer was stronger for mortality than for incidence [10]. Also, one study of 25(OH)D level in relation to colorectal mortality found a 72% lower risk in those with high 25(OH)D level (this study did not assess incidence of colorectal cancer) [56]; this risk reduction is greater than studies that have examined incidence as the endpoint. Since mortality is a function of both incidence rate and survival among those who develop cancer, this finding may suggest indirectly that vitamin D status may influence survival. Various lines of evidence from human studies suggest that vitamin D may be related to cancer progression. The evidence that vitamin D status may influence

colorectal cancer progression in humans in addition to effects on incidence is summarized in this section.

One clue that vitamin D may have late stage effects on tumor progression is from a large study in Norway that examined the influence of season of diagnosis on survival from colon cancer, as well as breast and prostate cancers. The premise was that in a place at high latitudes, like Norway, patients diagnosed with cancer during the summer and autumn months would have higher vitamin D levels compared to those diagnosed and treated during the winter months [57, 58]. This study included 12,823 men and 14,922 women with colon cancer. The period of observation was from 1964 to 1992. No significant seasonal variation in the incidence rate of colon cancers was noted, with about 25% of the cancers diagnosed in each season. However, death rates at 18 months, 36 months and 45 months were 20 to 30% significantly lower in those diagnosed in autumn months compared with those diagnosed in the winter months, with maximal benefit at 18 months. Vitamin D status is an obvious candidate to explain these differences in survival because of the known strong seasonal variation of solar UV-B radiation in Norway. However, other micronutrients related to fruits and vegetables may be consumed more in the summer months, so the association cannot necessarily be attributed to vitamin D. If related to vitamin D, this finding would lead to the speculation that a better vitamin D status at the time of diagnosis and treatment may enhance survival, possibly by optimizing the effects of treatment. Some animal models suggest that vitamin D analogues may improve tumor control by radiation treatment, partly by inducing apoptosis [59].

Prediagnostic 25(OH)D levels were examined in relation to mortality among 304 participants who developed colorectal cancer in the NHS and HPFS cohorts [60]. The cases were diagnosed with colorectal cancer from 1991 to 2002 and were then followed until 2005. This is follow-up of the dataset for which an overall protective association between 25(OH)D level and colorectal cancer risk was described previously [18]. In multivariate analyses, compared with those in the lowest quartile of 25(OH)D, cases in the highest quartile had a multivariate adjusted RR = 0.52 (95% CI: 0.29–0.94) for overall mortality and RR = 0.61 (95% CI: 0.31–1.19) for colorectal cancer-specific mortality. The cases that were diagnosed within 2 years after the blood was collected were excluded from the analysis to reduce the likelihood that the undiagnosed tumor, presumably with a worse prognosis, was not responsible for lowering 25(OH)D level (reverse causation). Further, the results persisted even after excluding patients diagnosed within 5 years of blood collection, further excluding reverse causation.

In a Japanese study of 257 colorectal cancer patients undergoing surgery with 39 deaths (30 of which were colorectal cancer-specific deaths), higher serum 25(OH)D levels were associated with better survival (P = 0.027) in multivariate analysis [61]. Since the cases were already diagnosed with cancer at the time of blood draw, potential reverse causation cannot be excluded. For example, sicker patients with a worse prognosis may have lower vitamin D levels due to less sun exposure or from direct metabolic effects of the disease, even after adjustment for other prognostic indicators.

An analysis of pre-diagnostic 25(OH)D and colorectal cancer survival was also done in the EPIC cohort [62]. The nested case-control analysis for incident cancer is described above [19]. Among 1,202 participants diagnosed with colorectal cancer between 1992 and 2003, 541 total deaths and 444 deaths due to colorectal cancer were identified over a mean follow-up time of 73 months. In multivariable analysis, higher 25(OH)D levels were associated with a statistically significant reduction in colorectal cancer-specific (P(trend) = 0.04) and overall mortality (P(trend) = 0.01). Cases with 25(OH)D levels in the highest quintile had an adjusted HR of 0.69 (95% CI: 0.50–0.93) for colorectal cancer-specific mortality and 0.67 (95% CI: 0.50–0.88) for overall mortality, compared with the lowest quintile.

In the NHS, HPFS and EPIC studies [60, 61], we cannot definitively determine the timing of the association as pre-diagnostic and post-diagnostic 25(OH)D levels are likely to be correlated. It is possible that poor vitamin D status before the diagnosis, when the tumor is developing, may affect some molecular characteristics of the cancer to make it behave more aggressively (i.e., worsens prognosis) independently of the 25(OH)D status after the diagnosis. Of course, beneficial effects of vitamin D on both pre-diagnostic and post-diagnostic stages are possible. To address a possible effect of post-diagnostic 25(OH)D, predicted 25(OH)D level (described above) was examined in relation to mortality among 1017 participants in the NHS and HPFS cohorts who were diagnosed with colorectal cancer from 1986 to 2004 [63]. Higher predicted 25(OH)D levels described above [21] were associated with a significant reduction in colorectal cancer-specific and overall mortality; compared with those with levels in the lowest quintile, participants with predicted 25(OH)D levels in the highest quintile had an adjusted RR = 0.50 (95% CI: 0.26–0.95) for cancer-specific mortality and 0.62 (95% CI: 0.42–0.93) for overall mortality. These associations persisted even after adjusting for pre-diagnostic predicted 25(OH)D level, suggesting the possibility that modifying the 25(OH)D level after diagnosis could have a beneficial effect on prognosis, though further study is needed.

The suggested relationship between vitamin D and colorectal cancer mortality and survival prompted an examination of the results from the WHI [22] and the United Kingdom randomized trials [55] for colorectal cancer-specific mortality. Based on small numbers, a suggestive lowered colorectal cancer mortality was observed in these trials for those randomized to vitamin D in both studies (also calcium in the WHI) and colorectal cancer mortality (pooled RR = 0.78; 95% CI: 0.52–1.17). The results from this post-hoc analysis are compatible with an effect of vitamin D in reducing cancer mortality independently of any effect on incidence. Because the doses and duration of the study were unlikely to be optimal for cancer prevention, the effect of vitamin D on cancer mortality should be stronger if this association is causal.

## SYNTHESIS AND CONCLUSIONS

Since a connection between vitamin D and colorectal cancer was first hypothesized in 1980, a number of studies have addressed this hypothesis using various approaches. Laboratory studies have provided strong evidence for a role of the vitamin D pathway on carcinogenesis. Intervention studies of vitamin D supplementation in humans have been relatively sparse and limited in scope. Epidemiologic studies based on other study designs have relatively consistently supported that vitamin D status influences colorectal cancer risk. Specifically, a higher level of circulating 25(OH)D has been associated with a decreased risk of colorectal adenoma and colorectal cancer. Further, among colorectal cancer patients, high exposure to vitamin D is associated with better survival. Ecologic studies from various populations show that those living in regions exposed to more solar UV-B radiation, and thus greater potential to synthesize vitamin D, have a lower risk of cancer incidence and especially mortality. Studies of vitamin D intake generally find a reduced risk of colorectal cancer and adenoma with higher intake, though the reduction is modest, compatible with the low levels of intake in most populations.

Because epidemiologic studies do not prove cause-and-effect, it is important to consider various criteria to consider the likelihood that observed associations are causal. For any observed association,

potential alternative explanations to a causal association are chance, various biases, and confounding. Based on the data summarized above, the association between higher 25(OH)D levels and greater solar UV-B exposure (based on region) and colorectal cancer is very unlikely due to chance or bias. The likelihood for potential biases such as detection bias or reverse causation vary by study design. The consistency of the association in studies of UV-B, vitamin D intake, measured or predicted 25(OH)D for adenoma, cancer incidence, and cancer survival cannot all be attributable to a single bias. For example, reverse causation is plausible for plasma-based studies when the tumor is diagnosed prior to blood draw or shortly after blood draw; however, the association is seen even many years after blood draw and for adenomas, which are unlikely to affect plasma 25(OH)D. Recall bias may occur in retrospective dietary studies, but unlikely to occur in prospective dietary studies or in studies based on biomarkers (i.e., 25(OH)D) or region.

Because the association between vitamin D status and lower colorectal cancer risk is unlikely due to chance or bias, confounding is the most important consideration. Confounding can be only completely excluded through randomization, but randomized trials to date are inadequate to resolve the question definitively. Arguably, body weight and physical activity are the most relevant potential confounding factors to consider because these influence 25(OH)D levels and are risk factors for colorectal cancer [64]. Individuals with greater adiposity tend to have lower 25(OH)D levels and less physically active individuals, probably due to less outdoor sun exposure, tend to have lower 25(OH)D levels. Obesity and physical inactivity also are risk factors for colorectal neoplasia, and thus could confound the inverse association between 25(OH)D and colorectal adenoma and cancer.

Although confounding by body mass index and physical activity could potentially explain the vitamin D – colorectal cancer link, several lines of evidence argue against this. (1) Most studies have adjusted for body mass index and physical activity level. Typically, adjustment did not appreciably alter the main results for 25(OH)D and colorectal cancer or adenoma risk. (2) The magnitude of the association between body mass index and physical activity and colorectal cancer is fairly modest [65, 66], and the stronger the association, the more likelihood for confounding. In fact, the association between body mass index and physical inactivity combined are much stronger for endometrial cancer than for colorectal cancer in women, suggesting that the potential for residual confounding (i.e. uncontrolled confounding by obesity and physical inactivity) is much stronger for endometrial cancer, but 25(OH)D appears unrelated to endometrial cancer [67]. This suggests epidemiologic studies can effectively control for the potential confounding effects of BMI and physical activity. (3) While lower body mass index and physical activity are consistently associated with lower risk of colon cancer, these are unlikely to be appreciably associated with rectal cancer risk [66]; thus, we would expect confounding to be much stronger for colon cancer than for rectal cancer but inverse associations with 25(OH)D appear even stronger for rectal cancer than for colon cancer. (4) The association is consistent in diverse populations across the world – confounding factors may be population-specific and unlikely to be the same across all populations. (5) While 25(OH)D level could potentially be confounded by physical activity and body mass index, these are not likely confounders of the studies based on region or vitamin D intake. Thus, while physical activity and adiposity are potentially confounding factors, the current evidence strongly suggests that these factors are unlikely to entirely account for the 25(OH)D – colorectal cancer relationship.

The totality of evidence, as summarized above, is supportive of a causal association between improved vitamin D status and lower risk of colorectal cancer. Further research can provide more evidence to definitively establish a causal relationship. Ongoing randomized trials of colorectal adenoma (ClinicalTrials.gov, Identifier: NCT00153816) and cancer (ClinicalTrials.gov, Identifier: NCT01169259) [68] could potentially demonstrate a causal association, though potential practical limitations of randomized trials (summarized above) could obscure a true association. Because suggestive evidence indicates a role of vitamin D in prognosis, efficiently designed studies of cancer survivors can be employed because the number of events to accumulate statistical power can be feasibly done in relatively small populations over several years (in contrast to primary prevention studies). Future epidemiologic analyses can better explore who might be more sensitive to vitamin D status; for example based on insulin, insulin-like growth factor and estrogen status [69], and the timing of the association. Studies can also better establish the optimal dose and blood level.

While further ongoing studies may better define the role of vitamin D on colorectal cancer, it is difficult to avoid formulating some recommendations based on current knowledge. A recent report from the Institute of Medicine [70] concluded that there was insufficient evidence to claim a definitive effect of vitamin D status on colorectal cancer risk (as well as non-skeletal endpoints in general). The Institute of Medicine set a desirable level of 25(OH)D as 20 ng/mL and recommended intakes of 600-800 IU per day for adults. Nonetheless, based on this current review, most studies found lower risk for colorectal cancer at levels above 20 ng/mL, with lower risk for levels of up to 40 ng/mL or even higher. Further complicating recommendations for vitamin D are that adhering to recommendations to limit sun exposure may lower 25(OH)D levels [71]. Thus, even if evidence for a role of vitamin D may not be deemed adequate for a definitive causal association (by the Institute of Medicine, for example), they may be sufficiently strong to warrant concern for potential harm caused by actions that inadvertently lower 25(OH)D status. Given the potential benefits from this vitamin against colorectal cancer, as well as other endpoints, further research should be a priority. Currently, it appears reasonable to target 25(OH)D levels in the range of 30-40 ng/mL, given potential benefits and low expectation of harm. Recommendations at this level are consistent with recent guidelines from the Endocrine Society [72] and other expert panels [73]. Maintaining high 25(OH)D levels may be especially important for higher risk groups for colorectal cancer, such as those with a positive family history of this cancer, those with a history of colorectal adenomas, and those with risk factors such as obesity.

## DISCLOUSURE

Part of information included in this article has been previously published in J Bone Miner Res. 2007 Dec;22 Suppl 2:V81-5.

## CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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