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Vitamin D for Prevention and Treatment of Colorectal Cancer: What is the Evidence?

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Abstract

Vitamin D insufficiency is highly prevalent in the U.S., particularly among colorectal cancer (CRC) patients. These low levels of vitamin D are concerning in light of increasing evidence that vitamin D may have health benefits beyond skeletal outcomes. Prospective observational studies suggest that higher vitamin D levels are associated with lower risk of incident CRC as well as improved survival in patients with established CRC, and randomized clinical trials are desperately needed to establish causality. Moreover, there remains a great need to improve prognosis for patients with CRC, and investigating vitamin D as a potential therapeutic modality is an attractive option in regards to safety and cost, particularly in this era of expensive and often toxic anti-neoplastic agents. In this review, the available published evidence on vitamin D's activity in CRC will be summarized, spanning preclinical, epidemiological, and clinical studies, and future research directions will be discussed.

Keywords

Colorectal cancer; vitamin D; 25-hydroxyvitamin D; calcitriol; vitamin D receptor; chemoprevention; randomized clinical trial; cancer epidemiology; nutrition

Introduction

Epidemiologic and scientific research indicates that diet and lifestyle factors have a significant influence on the development of colorectal cancer (CRC). Vitamin D, in particular, has been the subject of increasing academic and media interest due to abundant preclinical and observational data demonstrating its anti-neoplastic properties and potential association of higher levels with decreased risk of CRC and improved survival. Several sources of vitamin D exist, including synthesis in the skin following exposure to ultraviolet (UV)-B radiation from sunlight, food in the diet, and vitamin D supplements. These inputs of vitamin D₃ from the skin and intestinal tract are then converted to 25-hydroxyvitamin D [25(OH)D], the main circulating form, by 25-hydroxylase (CYP2R1) in the liver. Subsequently, 25(OH)D is converted to its active form, 1,25-dihydroxyvitamin D [1,25(OH)₂D] or calcitriol, in the kidney by 1 α -hydroxylase (CYP27B1). Binding of the

Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

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nuclear vitamin D receptor (VDR) by calcitriol leads to dimerization with the retinoic acid receptor (RXR) and binding of the complex to vitamin D response elements (VDREs) located in promoters and regulatory regions of target genes. While the most well-studied function of vitamin D is control of calcium and phosphate metabolism to maintain skeletal health, research over the last several years has revealed that 1 α -hydroxylase and VDR are actually present in most cells of the body,¹ including CRC cells,²⁻⁴ and that VDR also regulates target genes involved in induction of differentiation and apoptosis^{5,6} and inhibition of proliferation,⁷ angiogenesis,^{8,9} and metastatic potential.^{10,11} These anti-neoplastic actions of vitamin D, the presence of VDR diffusely in normal and cancer cells, and the extrarenal distribution of 1 α -hydroxylase all suggest that calcitriol can be synthesized locally within cancer cells to yield high concentrations within the tumor for intracrine and autocrine anti-cancer effects.

In light of the above hypothesis, it is concerning that 77% of Americans are currently vitamin D insufficient.¹² This rate may be rising due to increased use of sunscreen for skin cancer prevention, decreased outdoor activity, and higher prevalence of obesity, among other factors. In an attempt to address these concerns, the Institute of Medicine (IOM) and Endocrine Society each issued guidelines for vitamin D intake in 2011. While both groups agree that 25(OH)D is the best functional indicator of vitamin D status since it reflects all incoming sources of vitamin D, there is ongoing debate about what constitutes vitamin D deficiency. The IOM recommended a dietary allowance (RDA) of 600 IU/day for adults 19–70 years and 800 IU/day for adults >70 years to achieve circulating levels of 25(OH)D of 20 ng/mL¹³ (1 ng/mL=2.496 nmol/L), based on the minimal amount required to maintain skeletal health. They concluded that there was insufficient evidence to support targeting higher levels of vitamin D for chronic disease prevention and treatment. In contrast, the Endocrine Society recommended target 25(OH)D levels of 30 ng/mL, with levels <20 ng/mL considered deficient.¹⁴ These differences in the very definition of vitamin D deficiency and ongoing controversy regarding optimal levels highlight the importance of further understanding the biological mechanisms underlying vitamin D's role in CRC development and progression, and the critical need for rigorously designed, randomized clinical trials of chemoprevention and cancer treatment to establish vitamin D within the armamentarium of therapeutic options for CRC. In the review that follows, we will summarize the scientific, epidemiologic, and clinical progress to date in this field, as well as highlight important future research directions that will enhance our understanding of the relevant molecular pathways and potentially improve the prognosis of patients with CRC.

Vitamin D Activity in the Laboratory

Preclinical studies show that well-differentiated CRC cell lines have higher VDR expression,¹⁵ and the anti-proliferative effects of vitamin D seem to be greatest in cell lines that express high levels of VDR,¹⁶ consistent with the hypothesis described above that local production of calcitriol may be directly involved in cancer pathogenesis. Vitamin D inhibits growth and promotes differentiation of CRC cell lines and xenografts,^{8,17-20} and rats maintained on a diet enriched in calcitriol develop fewer intestinal tumors and metastases compared to control animals.^{11,21} Moreover, treatment of *Apc^{Min}* mice with vitamin D or a

synthetic analog reduces the size of intestinal adenomas,²² which is increased in *Apc^{+/-Min};VDR^{-/-}* mice.^{23,24}

Although abundant evidence implicates vitamin D in CRC biology, the exact underlying mechanisms and pathways remain unclear. Beyond direct effects on proliferation, cell differentiation, apoptosis, and metastatic potential mentioned above, several intriguing studies show that vitamin D can counteract aberrant WNT- β -catenin signaling, which is a known etiologic alteration in the development of CRC, by inhibiting nuclear translocation of β -catenin, sequestering β -catenin at the membrane, and increasing levels of extracellular WNT inhibitors DICCKOPF1 and DICKKOPF4.²⁵ Chronic inflammation is also a known risk factor for development and progression of CRC, and vitamin D exerts several anti-inflammatory effects through down-regulation of nuclear factor (NF)- κ B activity, increase in production of anti-inflammatory interleukin (IL)-10, and decrease in production of pro-inflammatory IL-6, IL-12, interferon- γ , and tumor necrosis factor (TNF)- α .²⁶ Moreover, vitamin D inhibits cyclooxygenase (COX) expression and decreases prostaglandin signaling, which not only suppresses inflammation but also decreases prostaglandin E₂-induced hypoxia-inducible factor (HIF)-1 α expression, leading to inhibition of angiogenesis.²⁷ Vitamin D also directly reduces expression of vascular endothelial growth factor (VEGF),²⁸ and *VDR*-null mice demonstrate increased expression of pro-angiogenic factors such as VEGF, HIF-1 α , and platelet-derived growth factor (PDGF).²⁹

Further mechanistic and genetic studies are required to elucidate the extensive and intricate interactions that seem to characterize vitamin D's role in CRC. Currently, the exact transcriptional targets of *VDR*, and even whether vitamin D acts directly on epithelial cells or within the tumor environment or both, remain unknown, and the influence of germline polymorphisms in vitamin D pathway genes on vitamin D metabolism and action are unclear. The role of vitamin D binding protein (VDBP, encoded by *GC*), to which 25(OH)D is bound in the circulation, is also unexplored, and it is uncertain whether total 25(OH)D levels or free or bioavailable 25(OH)D levels are the relevant substrate for vitamin D activity in cancer cells. Studies addressing these important questions are currently underway.

Vitamin D in CRC Prevention

Epidemiologic findings have overall paralleled the scientific observations above. Multiple prospective studies show that individuals with higher plasma levels of total 25(OH)D experience a significant reduction in risk of CRC compared to those with low plasma levels.^{30–35} In a meta-analysis of five epidemiologic studies, individuals with serum 25(OH)D level ≥ 33 ng/mL had a 50% lower risk of CRC compared to those with levels < 12 ng/mL ($P < 0.01$).³⁶ Another meta-analysis of 18 prospective studies showed that individuals with both higher dietary and supplemental intake of vitamin D, as well as higher plasma levels of 25(OH)D, have a significantly reduced risk of developing CRC,³⁷ with pooled relative risks (RRs) of 0.88 (95% CI, 0.80–0.96) and 0.67 (95% CI, 0.54–0.80), respectively. Moreover, a significant dose-response relationship was observed, with each 10 ng/mL increment in plasma 25(OH)D level associated with a RR of 0.74 (95% CI, 0.63–0.89) for CRC. Consistent with these findings, a report from the Third National Health and Nutrition Examination Survey (in which 16,818 participants were enrolled from 1988–1994 and

followed through 2000) demonstrated an inverse relationship between serum 25(OH)D levels and CRC mortality, with levels 32 ng/mL or higher associated with a 72% risk reduction (95% CI, 32%–89%), compared with levels <20 ng/mL (P trend=0.02).³⁸

Generally null findings for CRC incidence from the Women's Health Initiative (WHI), a randomized placebo-controlled trial of 400 IU vitamin D plus 1,000 mg a day of calcium in post-menopausal women, appear to contrast with epidemiologic data³⁴ (Table 1). However, relatively low-dose vitamin D supplementation (400 IU/day) may not confer a significant reduction in CRC risk, particularly with limited duration of follow-up. Indeed, the dose of 400 IU daily increased plasma 25(OH)D levels by only 2–3 ng/mL, whereas in most epidemiologic studies, the contrast between the high and low quintiles is 20 ng/mL.³⁶ Although the WHI provides important data, benefits of calcium and vitamin D may exist at doses and durations not assessed in that study. Interestingly, WHI participants who had the highest baseline levels of plasma 25(OH)D did experience a significant 60% reduction in CRC risk (P for trend=0.02). Thus, consistent with other studies, participants who managed to achieve higher 25(OH)D levels (through means other than the assigned vitamin D supplement) had a substantial reduction in CRC risk. Further, in the WHI, although the dose of vitamin D was clearly suboptimal, supplemental vitamin D did confer non-statistically significant reductions for CRC mortality (RR 0.82; 95% CI, 0.52–1.29; P =0.39), total cancer mortality (RR 0.89; 95% CI, 0.77–1.03; P =0.12), and total mortality (RR 0.93; 95% CI, 0.83–1.01; P =0.07). In addition, in a re-analysis of the study, vitamin D and calcium supplementation was found to decrease the risk of CRC among women who were randomized to the estrogen placebo arm of the trial (hazard ratio [HR] 0.71; 95% CI, 0.46–1.09).³⁹

Two other randomized, placebo-controlled trials of vitamin D supplementation designed primarily to look at fracture risk have reported results of secondary endpoints of cancer risk or mortality (Table 1). In the first trial, 2,686 subjects were randomized to receive 100,000 IU of vitamin D₃ versus placebo every four months,⁴⁰ and an age-adjusted RR of 0.62 (95% CI, 0.24–1.60) for colon cancer mortality was found with vitamin D compared to placebo (7 versus 11 colon cancer deaths, respectively). The second randomized trial evaluated the combination of vitamin D 1,100 IU/day + calcium 1,400–1,500 mg/day versus calcium alone versus placebo in 1,179 healthy post-menopausal women living in Nebraska, and demonstrated a 60% decrease in all-cancer risk (including CRC) in favor of the vitamin D-containing arm (P <0.03).⁴¹

Despite the fairly consistent evidence above, though, caution must be maintained, as most of the studies were observational in nature and CRC was a secondary end point in the randomized trials. Moreover, several large meta-analyses of plasma 25(OH)D concentrations and other cancer types have failed to find significant inverse associations,^{42,43} with some even reporting increased risk of prostate⁴⁴ and pancreatic cancer⁴⁵ with higher levels of 25(OH)D. Clearly, larger randomized placebo-controlled studies designed to primarily assess the impact of sufficiently high doses and duration of vitamin D on CRC risk with adequate sample size and power are urgently needed to determine whether vitamin D truly has chemopreventive activity. The VITamin D and Omega-3 Trial (VITAL) is such an ongoing phase III clinical trial that recently completed

its planned accrual of 25,875 healthy subjects aged 50 years and older ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01169259) identifier NCT01169259). VITAL involves a 2×2 randomization to vitamin D3 2,000 IU/day or fish oil 1 g/day compared to placebo for five years, with primary endpoints of cancer, cardiovascular disease, and stroke. Results are not anticipated for several years.

Vitamin D for CRC Treatment

Although multiple epidemiologic studies and clinical trials have been conducted to investigate the role of vitamin D (whether dietary intake, supplement use, or plasma 25(OH)D level) in CRC prevention, very few have been done to evaluate the influence of vitamin D status on survival of patients with established CRC. Moreover, cancer patients themselves often seek to understand what diet and lifestyle behaviors – beyond standard treatment with surgery, chemotherapy, or radiation – will improve their outcome. In 2001, an expert panel convened by the American Cancer Society recommended, “Properly conducted studies of the effect of nutrition and physical activity on the prognosis of cancer survivors are urgently needed, and should be a high priority for all academic and research funding agencies.”

Table 2 summarizes the six prospective epidemiologic studies that have been performed in CRC patients to try and address this knowledge gap. The first study evaluated 304 CRC patients in the Nurses’ Health Study (NHS) and Health Professionals Follow-Up Study (HPFS) who had blood available for analysis, and found that those with circulating pre-diagnostic 25(OH)D levels in the highest quartile had a multivariable HR for overall mortality of 0.52 (95% CI, 0.29–0.94; *P* trend=0.02).⁴⁶ The adjusted HR for CRC-specific mortality was 0.61 (95% CI, 0.31–1.19), but was not statistically significant (*P* trend=0.23), raising the alternative hypothesis that the improved outcome seen with higher concentrations of 25(OH)D could be explained by a beneficial effect of vitamin D on non-CRC-related conditions such as cardiovascular disease and diabetes. However, most of the deaths in the analysis of overall mortality were due to CRC, with only eight events attributable to cardiovascular or cerebrovascular events. Moreover, a significant relationship between 25(OH)D and CRC survival was subsequently confirmed in another study of 1,017 CRC patients in the NHS and HPFS, where higher post-diagnosis vitamin D scores calculated from known clinical determinants of vitamin D status were found to be significantly associated with both improved cancer-specific (adjusted HR 0.50; 95% CI 0.26–0.95; *P* trend=0.02) and overall survival (HR 0.62; 95% CI, 0.42–0.93; *P* trend=0.002).⁴⁷ Studies performed in other cohorts have also reproduced these findings, with HRs for overall and CRC-specific mortality that are very similar to the original reports.^{48–50} In sub-group analyses within the NHS and HPFS cohorts, the benefit of higher plasma 25(OH)D seemed greater in stage III and IV patients than stage I and II (adjusted HR 0.40 versus 0.90, respectively, comparing extreme quartiles).⁴⁶ To further test the benefit of vitamin D in advanced stage CRC, an analysis of plasma 25(OH)D levels drawn at study registration in 515 stage IV CRC patients enrolled in a completed, National Cancer Institute-sponsored clinical trial of palliative chemotherapy (North Central Cancer Treatment Group [NCCTG] 9741) was performed.⁵¹ This cohort of metastatic CRC patients had extremely low circulating concentrations of 25(OH)D at baseline, with a median level of 20.0 ng/mL. Indeed, only 10% of the study population had levels ≥ 33 ng/mL, the threshold believed to be

required for a protective effect of vitamin D on CRC risk.³⁶ Possibly as a result of this skewed distribution of 25(OH)D, no significant association was detected between higher 25(OH)D levels and improved outcome in the NCCTG 9741 study. However, in a post-hoc exploratory subgroup analysis, patients who were randomized to receive FOLFOX chemotherapy and had 25(OH)D levels in the highest quartile showed a multivariable HR of 0.64 (95% CI, 0.45–0.90; *P* trend=0.003) for overall mortality compared to patients on FOLFOX with levels in the lowest quartile.

While the data above are intriguing and generally consistent in suggesting a potential role for vitamin D in treatment of CRC, the possibility of reverse causation must be considered. A recent large meta-analysis of observational studies and randomized clinical trials of vitamin D status and multiple health outcomes, including cancer survival, was published in *The Lancet Diabetes & Endocrinology*.⁵² The authors noted that while prospective observational studies consistently showed decreases in cardiovascular disease, serum markers of cardiovascular disease and inflammation, diabetes, infectious diseases, and cancer incidence and mortality with higher concentrations of 25(OH)D, results of randomized intervention studies did not corroborate these findings. They hypothesized that low circulating 25(OH)D may therefore be a consequence of ill health, rather than the cause. Furthermore, systemic and local inflammation, which characterizes all of the conditions listed above, may serve as the underlying mechanism by which disease leads to low vitamin D concentrations. Although certainly plausible, results from the analysis of plasma 25(OH)D levels and CRC survival nested within the NHS and HPFS discussed above argue against this hypothesis by trying to control for reverse causation.⁴⁶ In that paper, CRC patients who received their cancer diagnosis within two years of 25(OH)D measurement were excluded from the analysis, in case the presence of occult illness and cancer resulted in lower 25(OH)D values. Indeed, the significant relationship between 25(OH)D levels and survival was even preserved in sensitivity analyses that excluded patients who were diagnosed within five years of 25(OH)D assessment. However, further exploration of the relationship between vitamin D, inflammation, and CRC is clearly warranted.

As with chemoprevention, definitive evidence of vitamin D efficacy in treatment of CRC can only be obtained from rigorously-designed, randomized clinical trials. Given the prevalence of vitamin D deficiency and insufficiency among metastatic CRC patients, the hypothesis-generating finding of a greater benefit of vitamin D among advanced stage patients, and preclinical studies revealing potential synergy between vitamin D and cytotoxic drugs such as platinum^{53–56} and 5-FU,⁵⁷ a randomized, double-blind phase II trial of FOLFOX + bevacizumab chemotherapy with standard-dose (400 IU/day) versus higher-dose vitamin D3 (4,000 IU/day) in previously untreated stage IV CRC patients has been designed and is currently enrolling patients at multiple centers across the U.S. (ClinicalTrials.gov identifier NCT01516216). The primary endpoint is progression-free survival, and accrual of approximately 120 patients is planned. Serial plasma and DNA samples and archival tumor tissue will be collected from all patients and banked for future correlative studies to further elaborate on underlying molecular and genetic mechanisms within the context of a human intervention study.

Conclusion

The potential to modulate the development and progression of CRC through nutritional and lifestyle factors such as vitamin D is very real, but insufficiently studied; a clinical and translational approach that steps beyond purely observational studies is required to establish causality. In addition, elucidation of a biological mechanism underlying these clinical findings would enhance the acceptability of vitamin D as critical for cancer prevention and treatment. In this era of expensive and often toxic anti-neoplastic drugs, vitamin D represents an attractive treatment option for patients and oncologists with respect to both safety and cost, and improving understanding of vitamin D in CRC could potentially shift the paradigm in managing this disease.

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Conflict of Interest

Kimmie Ng has received compensation from Genentech, Inc. for serving on an advisory board, and has received support through a grant (as well as non-financial support) from Pharmavite, LLC.

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Table 1Randomized, placebo-controlled trials of the effect of vitamin D supplementation on cancer risk or mortality^a

	WOMEN'S HEALTH INITIATIVE³⁴	NEBRASKA STUDY⁴¹	UK STUDY⁴⁰
Study population	Healthy post-menopausal women	Healthy post-menopausal women	Elderly men and women
Age of participants (years)	50–79	>55	65–85
Number of patients	36,282	1,179	2,686
Date of enrollment	1993–1998		1996–1997
Intervention arm(s)	Vitamin D3 400 IU/day + Calcium 1,000 mg/day	Vitamin D3 1,100 IU/day + Calcium 1,400–1,500 mg/day or Calcium 1,500 mg/day alone	Vitamin D3 100,000 IU every 4 months
Duration of supplementation	Average 7.0 years	4 years	5 years
Compliance with protocol treatment ^b	60%	85.7%	76%
Duration of follow-up	Mean 7.0 years	4 years	5 years
Cancer endpoint	Incidence of pathologically-confirmed colorectal cancer	All incident cancers	Mortality from colon cancer
Effect estimate	HR 1.08 (95% CI, 0.86–1.34)	RR 0.402 (95% CI, 0.20–0.82) ^c	RR 0.62 (95% CI, 0.24–1.60)
<i>P</i> value	0.51	0.013 ^c	0.33

^aCancer risk or mortality were secondary endpoints in all of the included trials.^bCompliance defined as taking at least 80% of study medication.^cFor the vitamin D3-containing arm.

Table 2

Prospective studies of 25-hydroxyvitamin D3 [25(OH)D] and survival in colorectal cancer (CRC) patients

	No. CRC patients	Stages included	Exposure	Time of exposure assessment	Overall mortality	CRC-specific mortality
NHS/HPFS-plasma ⁴⁶	304	I-IV	Plasma 25(OH)D	Mean 6 years before CRC diagnosis	HR 0.52 ^a (95% CI, 0.29–0.94) <i>P</i> trend=0.02	HR 0.61 ^a (95% CI, 0.31–1.19) <i>P</i> trend=0.23
NHS/HPFS-score ⁴⁷	1,017	I-IV	Vitamin D score ^b	Within 1–4 years after CRC diagnosis	HR 0.62 ^a (95% CI, 0.42–0.93) <i>P</i> trend=0.002	HR 0.50 ^a (95% CI, 0.26–0.95) <i>P</i> trend=0.02
Japanese cohort ⁴⁸	257	I-IV	Serum 25(OH)D	At time of surgery	HR 0.91 ^c (95% CI, 0.84–0.99) <i>P</i> trend=0.027	HR 0.98 ^c (95% CI, 0.89–1.08) <i>P</i> trend=0.67
NCCTG 9741 cohort ⁵¹	515	IV	Plasma 25(OH)D	Prior to starting first-line chemotherapy	HR 0.94 ^a (95% CI, 0.72–1.23) <i>P</i> trend=0.55	--
JANUS cohort ⁵⁰	52	I-IV	Serum 25(OH)D	Median 37 days after CRC diagnosis	HR 0.40 (95% CI, 0.10–1.60) <i>P</i> trend=0.23	HR 0.20 (95% CI, 0.04–1.10) <i>P</i> trend=0.16
EPIC cohort ⁴⁹	1,202	I-IV	Serum 25(OH)D	Mean 3.8 years before CRC diagnosis	HR 0.67 ^a (95% CI, 0.50–0.88) <i>P</i> trend<0.01	HR 0.69 ^a (95% CI, 0.50–0.93) <i>P</i> trend=0.04

NHS = Nurses' Health Study; HPFS = Health Professionals Follow-Up Study; NCCTG = North Central Cancer Treatment Group; EPIC = European Prospective Investigation into Cancer and Nutrition.

^a Effect estimate comparing the highest quantile to the lowest quantile.

^b The vitamin D score predicts a 25(OH)D level based on known clinical predictors of vitamin D status: race (surrogate for skin pigmentation), geographic region of residence (surrogate for UV-B exposure), dietary and supplemental vitamin D intake, body-mass index, and physical activity.

^c Effect estimate per 1 ng/mL increase in 25(OH)D.