Vitamin D and Colorectal Cancer: Current Perspectives and Future Directions

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Vitamin D is considered to be the main mediator of the beneficial effects of sun exposure. In humans, highest expression of Vitamin D receptors is found in the intestinal tract. In addition, 1α,25-dihydroxyvitamin D3 (or calcitriol), the most active Vitamin D metabolite, plays important homeostatic roles in the intestine, particularly calcium absorption. Vitamin D deficiency is defined as a serum 25-hydroxyvitamin D [25(OH)D] level of < 20 ng/mL. Previous studies show that higher circulating 25(OH)D levels are associated with reduced risk of colorectal cancer (CRC) and improved survival. Most research to date has been conducted in animals, specifically mice. Although human studies have a limited number of participants, one study recruiting a large cohort of patients with advanced or metastatic CRC revealed that higher plasma 25(OH)D levels are associated with improved overall and progression-free survival. However, the effects of Vitamin D supplementation on incidence and mortality of CRC remain inconclusive. Although Vitamin D may help to prevent cancer, there is a paucity of research demonstrating conclusively that Vitamin D alters prognosis after chemotherapy. Here, we review the mechanisms by which Vitamin D affects CRC, as well as the results of clinical, epidemiological, and human intervention studies. We also discuss current perspectives and future directions regarding Vitamin D and CRC.

Key Words Vitamin D, 25-Hydroxyvitamin D, Vitamin D deficiency, Colorectal neoplasms

INTRODUCTION

In the past decade, colorectal cancer (CRC) has become the third most common cancer worldwide, and the second leading cause of cancer-associated death [1,2]. In 2020, it was estimated that about 10% of global cancer incidence, and about 9.4% of cancer-related deaths, could be attributed to CRC [2]. Risk factors for CRC include sex, age, race, and genetic factors [2-6]. Exposure to environmental risk factors, such as the shift to a modern lifestyle and a Westernized diet, plays a very important role in the increasing global incidence of CRC [7]. Epidemiological and observational studies have identified CRC as the neoplasm most commonly associated with Vitamin D deficiency [8]. However, design, data analysis and interpretation of some epidemiological and prospective interventional clinical studies are controversial.

Vitamin D is the principal mediator of the beneficial effects of sun exposure [9]. The human body manufactures 80%–90% of its Vitamin D via UV exposure, and 10%–20% is from nutrients absorbed in the small intestine. Food sources include salmon, mackerel, eel, tuna, eggs, milk, yogurt, and mushrooms [10]. Vitamin D is metabolized in the liver as Vitamin D3 (cholecalciferol), with 25-hydroxyvitamin D [25(OH)D] excreted by the kidney [11]. The small intestine is the organ showing highest expression of Vitamin D receptors (VDR). Furthermore, 1α,25-dihydroxyvitamin D3 [1,25(OH)$_2$D$_3$] (also called calcitriol), the most active Vitamin D metabolite, has many important homeostatic actions. Serum levels of 25(OH)D formed upon exposure to sunlight never exceed 60 ng/mL [12]. According to the Mayo Clinic reference ranges, total serum 25(OH)D levels range from 30–80 ng/mL. Vitamin D insufficiency is defined as a 25(OH)D level of < 30 ng/mL,

whereas deficiency is defined as < 20 ng/mL (values < 10 ng/mL are considered extremely low). By contrast, a 25(OH)D level of ≥ 80 ng/mL is defined as possible toxicity [13].

Vitamin D deficiency is pandemic and currently one of the major global health issues. Vitamin D deficiency affects. more than 1 billion children and adults worldwide. Pregnant women, people of color (Black, Hispanic, and anyone with increased skin melanin pigmentation), obese people, and people who avoid direct sun exposure are at high risk [14]. Vitamin D deficiency is prevalent in Europe, China, India, the Middle East, Southeast Asia, Central and South America, and Africa, where foods are not fortified with Vitamin D. It is estimated that approximately 30% of children and 60% of adults worldwide are either Vitamin D deficient or insufficient [15]. The major cause of deficiency is lack of sufficient sun exposure. Skin coloration and aging are two primary physiological factors underlying low levels of Vitamin D production. Very few foods contain Vitamin D as natural ingredient [16,17].

In the remainder of this article, we review the role of Vitamin D in CRC, as well as the results of clinical studies. We also discuss epidemiological and human intervention studies, and possible reasons why evidence for an effect of Vitamin D supplementation remains inconclusive.

METABOLISM OF VITAMIN D IN CRC

Calcitriol metabolism

Calcitriol has many important homeostatic actions in the small intestine. Of the four forms of Vitamin D (Vitamin D3, Vitamin D2 [ergocalciferol], calcidiol, and calcitriol), Vitamin D3 is almost exclusively produced after exposure to sunlight, while Vitamin D2 is obtained from plants. They are transported to the liver and converted to calcidiol, the active form of Vitamin D in the body. Calcidiol is converted to calcitriol mainly in the kidney (Fig. 1).

The three cytochrome P450s (CYPs) involved in Vitamin D metabolism are *CYP27A1*, *CYP27B1*, and *CYP24B1*. In the liver, *CYP27A1* hydroxylates Vitamin D to 25(OH)D. Then,

CYP27B1 catalyzes conversion of 25(OH)D to its active form, calcitriol, in the kidney; the latter exerts a biological effect in humans. When calcitriol levels increase, *CYP24A1* converts calcitriol to its water-soluble inactive form, calcitroic acid. *CYP24A1* is highly expressed in cancer tissues, where it promotes cancer progression by neutralizing Vitamin D, which may have antitumor effects. Knockdown of *CYP24A1* and calcitriol may be a potential treatment for carcinoma cells [18-20].

Effects of calcitriol in the colon

The physiological role of Vitamin D is regulation of calcium and phosphate metabolism, which accounts for its broader range of biological functions, including potential anti-neoplastic effects. Calcitriol, a biologically active form of Vitamin D, plays an important role in calcium metabolism and bone mineralization [21]. VDRs are highly expressed in the gut, mediate the biological actions of the calcitriol, and play a crucial role in immune regulation, proliferation, and intestinal homeostasis.

Calcitriol promotes differentiation of epithelial cells to maintain the intestinal epithelial barrier [8]. It also contributes to intestinal homeostasis by exerting complex effects on immune cells, inducing expression of xenobiotic detoxifying enzymes, and regulating the gut microbiota. In CRC, calcitriol inhibits angiogenesis as well as proliferation, and invasion of carcinoma cells, and sensitizes them to apoptosis.

The composition of the gut microbiome of colorectal cancer patients and healthy people is different. The human colon microbiota has many *Bacteroidetes* and *Bifidobacterium* species, which may have beneficial roles in maintaining health, whereas CRC patients have disease-associated gut microbes, such as *Parvimonas micra*, *Fusobacterium nucleatum*, *Porphyromonas,* and *Bacteroides fragilis* [22,23]. In a mouse model, Vitamin D deficiency aggravated the deterioration of enteritis and intestinal cancer, and these conditions were improved after Vitamin D supplementation. Further, Vitamin D plays a role in the regulation of colon barrier integrity
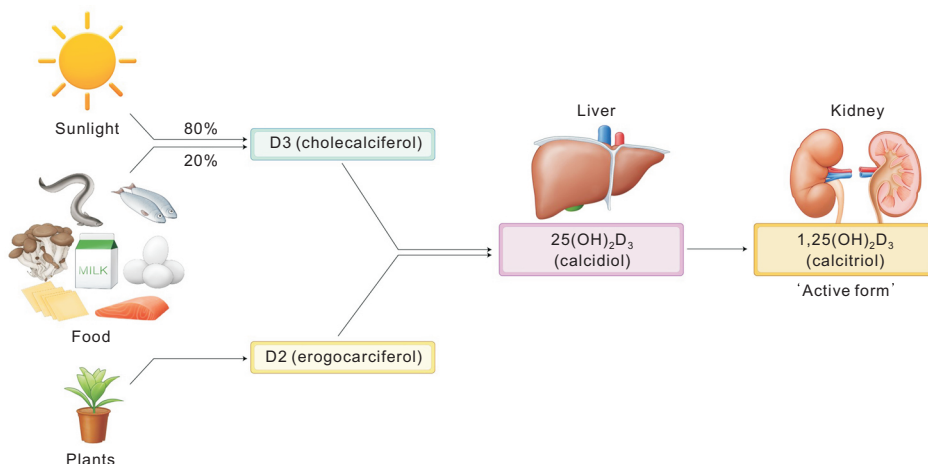
Figure 1. The four major forms of Vitamin D; Vitamin D3 (cholecalciferol), Vitamin D2 (ergocalciferol), calcidiol, and calcitriol.

mediated by the probiotic intestinal bacterium, *Akkermansia muciniphila* [24]. According to a systematic Review of Human Studies, Vitamin D supplementation and/or an elevated 25(OH)D level induce(s) a shift in bacterial composition and can affect the species diversity. Vitamin D supplementation upregulates *Firmicutes, Actinobacteria* and *Bacteroidetes* phyla, and downregulates *Veillonellaceae* and *Oscillospiraceae* families, in the *Firmicutes* phylum [25].

MECHANISMS OF VITAMIN D ACTION IN CRC

Calcitriol interferes with the growth and invasiveness of carcinoma cells by promoting differentiation of epithelial cells; these cells act as a barrier, interfere with migration of carcinoma cells through the extracellular matrix, maintain homeostasis by facilitating normal immune responses, and regulate the composition of the microbiota. Calcitriol promotes apoptosis by regulating the expression of genes related to apoptosis [26]. Calcitriol also upregulated the differentiation of colon carcinoma cells by inducing E-cadherin and the inhibition of β-catenin signaling [27]. Calcitriol acts by binding to the high-affinity VDR, expression of which is increased in hyperplastic polyps during the early stages of tumorigenesis, but falls at the later stages of neoplasia [28].

Many mechanistic studies demonstrate that calcitriol inhibits proliferation and promotes epithelial differentiation of VDR-expressing human colonic carcinoma cell lines. A key action underlining this effect is multilevel inhibition of Wnt/β-catenin signaling, abnormal activation of which in colon epithelial cells triggers and promotes CRC [29].

Anti-proliferative activity
Calcitriol inhibits proliferation of carcinoma cells by inducing G1 cell cycle arrest which is attributable to upregulation of the cell cycle inhibitors $p21^{WAF1/CIP}$ and $p27^{KIP}$. The promotor region of *p21* contains Vitamin D response elements; therefore, calcitriol can regulate transcription of *p21* gene directly. TGF-β, which inhibits epithelial proliferation, is disrupted in CRC. Calcitriol induces expression of the TGF-β1 receptor to restore TGF-β sensitivity [30-33].

Regulation of the Wnt signaling pathway
The Wnt/β-catenin pathway is activated primarily during embryonic development; however, it also contributes to homeostasis of other tissues. The pathway is activated aberrantly in several carcinomas, including CRC, and is implicated in expression of several genes that contribute development and progression carcinogenesis. Active Vitamin D inhibits Wnt/β-catenin signaling via multiple pathways. CRC contains approximately 4.5 times more Wnt/β-catenin-stained cells than normal colon cells. Activation of Wnt/β-catenin occurs early in CRC, and in most cases of CRC, one to two genes in this pathway harbor mutations. The pathway is activated by mutation of genes encoding *APC*, *CTNNB1*/β-catenin, or *AXIN*, or by dysregulation of the Wnt receptor. As a result, β-catenin accumulates in the cytoplasm and nucleus, while expression of target genes *c-MYC*, *TCF1*, *LEF1*, as well as transcription of *AXIN2*, *PPARδ*, *CD44*, *ENC1*, and *EPHB2*, is increased. This appears to be dependent on expression of the VDR. Calcitriol promotes VDR/β-catenin binding, reduces the amount of β-catenin bound to TCF, induces expression of the *CDH1* gene encoding E-cadherin (which sequesters β-catenin), and increases extracellular expression of the Wnt inhibitor *DKK-1* [19,34-38].

Anti-angiogenic effects
VEGF is required for formation of the new blood vessels necessary for growth and dissemination of solid tumors. Calcitriol inhibits transcription and expression of Hypoxia-inducible factor-1 (HIF-1), and decreases expression of VEGF. Calcitriol triggers antiangiogenic effects through NF-κB signaling, and the nuclear proteins fork into headbox M1 (FOXM1) and Dickkopf 4 (DKK4). It also inhibits interleukin-8 (IL-8), an important angiogenic factor [39].

Apoptoic effects
Vitamin D affects apoptosis of CRC cells. Calcitriol suppresses expression of anti-apoptotic proteins such as BCL-2, BAG1, and BCL-XL, and induces expression of pro-apoptotic proteins BAX, BAK, and BAD. Calcitriol facilitates the release of cytochrome *c* from mitochondria which leads to activation of caspases 3 and 9 [26]. Colonic epithelial VDR inhibits apoptosis of epithelial cells by downregulating p53 upregulated modulator of apoptosis (PUMA), an important proapoptotic regulator that protects the mucosal barrier and reduces inflammation [40].

Chemopreventive role of Vitamin D in CRC
Vitamin D plays role chemoprevention of CRC [41]. It exerts anti-proliferative effects in human colon cells by inducing cell cycle arrest through $p21^{WAF1/CIP}$ and $p27^{KIP}$; it restores sensitivity to TGF-β signaling, interferes with the synthesis of growth factors, regulates differentiation, and exerts anti-cancer effects by inducing apoptosis. Calcium intake reduces the risk of CRC. Vitamin D exerts an anti-inflammatory effect by interfering with the synthesis of prostaglandins, stress-activated kinase signaling, and production of proinflammatory cytokines, as well as acting as a potent inhibitor of tumor cell-induced angiogenesis, inhibiting stress-activated kinase signaling, and inducing mitogen-activated protein kinase, all of which have an anti-cancer effect. Vitamin D-activated maternally expressed gene 3 (*MEG3*) suppresses glycolysis in CRC by promoting degradation of *c-Myc* [8,37,42,43]. The anti-cancer effects mediated by Vitamin D signaling are illustrated in Figure 2.
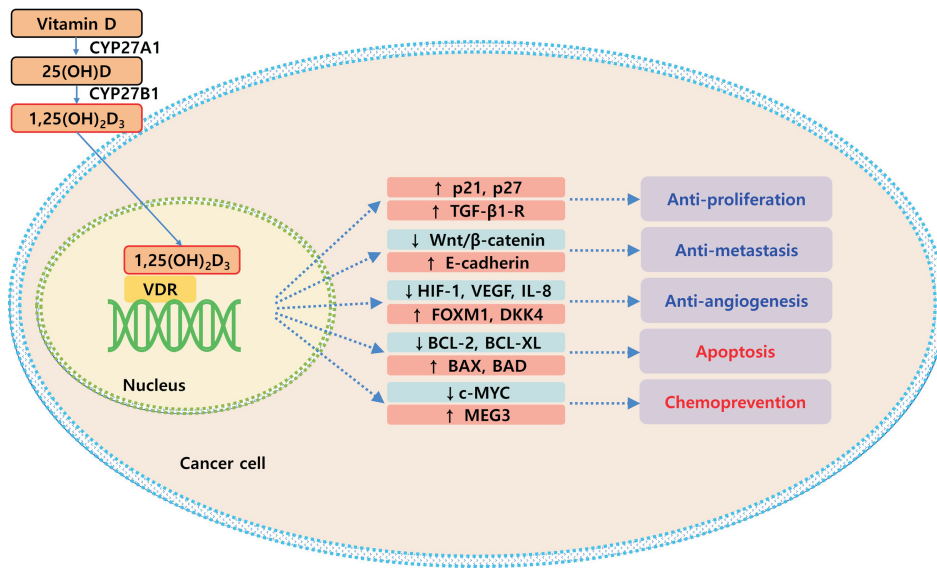
Figure 2. Anti-cancer effects mediated by Vitamin D signaling pathways.

THERAPEUTIC ROLE OF VITAMIN D IN CRC

The numerous antitumor effects of Vitamin D suggest that supplementation would be effective in reducing the occurrence, size, or recurrence of tumors. However, many studies show that supplementation with Vitamin D does not in itself have a statistically significant clinical effect. The VITamin D and OmegA-3 TriaL (VITAL) study showed no association between Vitamin D supplementation and reduced risk of colorectal adenoma (CRA) or serrated polyps [44]. However, the COLON (COlorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that may influence colorectal tumor recurrence, survival and quality of life) study showed that supplementation with ≥ 50 nmol/L, coupled with a high magnesium intake (median split) (hazard ratio [HR], 0.53; 95% confidence interval [CI], 0.31–0.89), lowered the mortality in CRC patients [45]. MDL-811, a small-molecule activating sirtuin 6 (SIRT6) synergically enhances the anti-CRC effect of Vitamin D both in vitro and in vivo [46]. Promoter methylation of secreted frizzled-related protein, an Wnt antagonist was decreased in patients who had higher levels of Vitamin D. Maintaining a high concentration of Vitamin D, therefore, may increase the response to neoadjuvant treatment [47].

OBSERVATIONAL STUDIES ON THE EFFECTS OF VITAMIN D IN CRC

The hypothesis that Vitamin D protects against colon cancer arose from a study on the geographic distribution of colon cancer mortality in the US. The study by Garland [48] showed an inverse association between annual mean daily solar radiation and age-adjusted rates of colon cancer-related. The result of study is considered to support the hypothesis that there is a relationship between Vitamin D and CRC.

Serum 25(OH)D levels and the risk of CRC

It is generally accepted that 25(OH)D levels of > 30 ng/mL (75 nmol/L [1 ng/mL = 2.5 nmol/L]) are sufficient, with 20–29 ng/mL regarded as insufficiency and < 20 ng/mL (50 nmol/L) defined as Vitamin D deficiency [49,50]. Ng et al. [51] measured serum 25(OH)D levels prospectively in 515 patients with stage IV CRC, all of whom were enrolled in a randomized controlled trial (RCT) of chemotherapy (the N9741 trial). Subgroup analysis revealed that Vitamin D deficiency (50% had < 20 ng/mL; 82% had < 30 ng/mL) was highly prevalent in all patients with stage IV CRC. The researchers also demonstrated that median serum 25(OH)D levels in black patients were lower than those in white patients and those of other races (10.7 vs. 21.1 vs. 19.3 ng/mL, respectively; $P = 0.001$), and also lower in females than in males (18.3 vs. 21.7 ng/mL, respectively; $P = 0.0005$) [51]. In general, black people show lower cutaneous synthesis of Vitamin D in response to solar radiation [52], and the higher levels of adipose tissue in females than in males may result in lower 25(OH)D levels [53]. A recent large cohort study by Yuan et al. [54] reported Vitamin D deficiency (63% had < 20 ng/mL; 94% had < 30 ng/mL) in 1,041 patients with previously untreated advanced or metastatic CRC.

Many observational studies have examined the association between serum 25(OH)D levels and the incidence of CRC; the evidence for inverse association between serum 25(OH)D levels and CRC incidence seems relatively strong. A nested case-control study from the Women's Health Study based on 274 CRC cases and 274 controls revealed that the mean serum 25(OH)D level was lower in cases than in controls (21.9 vs. 23.9 ng/mL, $P = 0.01$), and that serum 25(OH)D levels were inversely associated with CRC (quartile 4 vs. quartile 1; odds ratio [OR], 0.45; 95% CI, 0.25–0.81; P for trend=0.02) [55].

A recent meta-analysis of 17 cohorts comprising 5,706 CRC cases and 7,107 controls with a wide range of serum

25(OH)D levels shows that deficiency of 25(OH)D (< 20 ng/mL) is associated with a 31% higher CRC risk (relative risk [RR], 1.31; 95% CI, 1.05–1.62), and that sufficiency (30–35 or 35–40 ng/mL) is associated with 19% (RR, 0.81; 95% CI, 0.67–0.99) and 27% (RR, 0.73; 95% CI, 0.59–0.91) lower risk [56]. In this analysis, higher serum 25(OH)D levels showed a significant association with female, but not male, sex for lowering the risk of CRC [56]. Another meta-analysis of 30 studies from 1989 to 2019 also indicates a negative association between serum 25(OH)D levels and CRC risk (RR, 0.68; 95% CI, 0.60–0.78); a subgroup analysis showed that this difference was significant in females (RR, 0.57; 95% CI, 0.43–0.75) but not in males (RR, 0.91; 95% CI, 0.75–1.11). One interesting subgroup analysis in this study revealed that higher serum 25(OH)D levels are significantly related to a lower incidence of CRC in European and American (RR, 0.67; 95% CI, 0.58–0.76), but not in Asian, populations (RR, 0.84; 95% CI, 0.58–1.22) [57]. However, a meta-analysis of eight case-control studies from Asian countries (a total of 2,916 cases and 6,678 controls; mean serum 25(OH)D level = 20.21 ± 7.92 ng/mL [3.65–36.5 ng/mL]) revealed that the incidence of CRC was lower in the highest category than in the lowest (OR, 0.75; 95% CI, 0.58–0.97), although this meta-analysis identified heterogeneity among the studies (I^2 = 53.9%; P = 0.034) because the definition of serum 25(OH)D categories differed across studies [58]. A dose-response meta-analysis shows that an incremental increase in serum Vitamin D level of 16 ng/mL yields a 21% reduction in the incidence of CRC (OR, 0.79; 95% CI, 0.64–0.97) [58], suggesting that a higher serum 25(OH)D concentration is associated with a reduced risk of CRC in Asian countries, similar to Western populations.

With respect to the association between serum 25(OH)D levels and colorectal polyps, several meta-analyses of observational studies show an inverse association between serum 25(OH)D levels and CRA in both Western and Asian populations [57,59-62]. A recently published meta-analysis of 23 studies showed that the incidence of CRA correlated inversely with serum 25(OH)D levels (RR, 0.80; 95% CI, 0.71–0.89) [57]. This inverse association was significant in females (RR, 0.63; 95% CI, 0.45–0.89) but not in males (RR, 0.89; 95% CI, 0.68–1.16), and in European and American (RR, 0.82; 95% CI: 0.75–0.91), but not Asian (RR, 0.67; 95% CI, 0.40–1.14), populations [57].

Serum 25(OH)D levels and survival of CRC patients

Previous observational studies suggest that higher serum 25(OH)D levels are associated with reduced risk of CRC. However, the effect and role of Vitamin D on disease progression and outcome remain unknown. A meta-analysis that included five notable prospective cohort studies from 2008–2012 (a total of 2,330 CRC patients with a follow-up of 2.7–24 years) estimated the association between serum 25(OH)D levels and survival [63]. The analysis included patients from four Western studies and one Asian study [51,64-67]. The highest category was associated with much lower overall (HR, 0.71; 95% CI, 0.55–0.91) and disease-specific (HR, 0.65; 95% CI, 0.49–0.86) mortality of patients than the lowest category despite the heterogeneous 25(OH)D categorization among individual studies [63].

A concentration-response sub-analysis revealed that an increase in the serum 25(OH)D level by 8 ng/mL was associated with reduced overall (HR, 0.91; 95% CI, 0.81–1.01) and disease-specific (HR, 0.90; 95% CI, 0.84–0.97) mortality [63].

Yuan et al. [54] measured serum 25(OH)D levels prospectively in 1,041 patients with previously untreated advanced or metastatic CRC; all patients were participating in a phase 3 RCT (CALGB/SWOG 80405) of chemotherapy. They showed that the highest quintile (≥ 24.1 ng/mL) was associated with improved overall (HR, 0.66; 95% CI, 0.53–0.83) and progression-free (HR, 0.81; 95% CI, 0.66–1.00) survival when compared with the lowest quintile (≤ 10.8 ng/mL) [54]. This finding was consistent regardless of prognostic factors. Two recent meta-analyses by Wu et al. [68] and Huang et al. [57] have been published. The former analyzed 17,770 CRC patients from 17 studies and showed inverse association between high versus low 25(OH)D levels and overall (HR, 0.64; 95% CI, 0.55–0.72) and CRC-specific (HR, 0.65; 0.56–0.73) survival. A pooled concentration-response analysis revealed a 7% reduction (HR, 0.93; 95% CI, 0.90–0.95) in all-cause mortality and a 12% reduction (HR, 0.88; 95% CI: 0.84, 0.93) in CRC-specific mortality per 8 ng/mL increment in the serum 25(OH)D level [68]. The latter, which included 12 studies, also demonstrated that higher serum 25(OH)D levels lead to more favorable results regarding overall (HR, 0.69; 95% CI, 0.61–0.78) and CRC-specific survival (HR, 0.64; 95% CI, 0.56–0.73) [57].

Interventional studies on the effects of Vitamin D in CRC

Vitamin D supplements comprise either Vitamin D3 or Vitamin D2. Vitamin D2 has less than one-third the potency and a shorter duration of action than Vitamin D3 with respect to increasing serum 25(OH)D levels [69]. Previous studies suggest that Vitamin D3 is better than Vitamin D2 for patients who require Vitamin D supplementation [69-71]. A Cochrane review showed that Vitamin D2 did not confer a mortality benefit [72]. Vitamin D supplements vary in concentration from 400 international units (IU) (low dose) to 5,000 IU (high dose). To prevent Vitamin D deficiency, the Institute of Medicine recommends a daily intake of 600 IU per day, with a maximum recommended intake of 4,000 IU per day [73]. For those aged over 70 years, 800 IU is recommended. The Endocrine Society's recommendation is a daily allowance of 1,500–2,000 IU, with an upper limit of 10,000 IU for adults [73].

Previous observational studies show that higher serum

25(OH)D levels are associated with CRC incidence and survival rates; however, observational studies cannot address whether higher 25(OH)D levels cause a reduction in CRC incidence and/or improve outcomes. Animal studies show that Vitamin D analogs (BGP-13 and BGP-15) inhibit growth and induce apoptosis of human HT-29 tumor xenografts [74]. Also, adjuvant treatment using Vitamin D analogs PRI-2191 and PRI-2205 plus chemotherapy shows stronger antitumor effects than chemotherapy alone (5-fluorouracil, irinotecan, or oxaliplatin) [75,76].

Vitamin D intake and the risk of CRC

From 1996–1997, Trivedi et al. [77] conducted an RCT of 2,686 participants (2,037 males and 649 females) over the age of 65 years to determine the effect of Vitamin D on fractures and mortality; patients took one capsule (100,000 IU) of Vitamin D3 or placebo every 4 months for 5 years. After 5 years, the mean 25(OH)D levels in the treatment and placebo groups were 29.7 ng/mL (± 8.3) and 21.4 ng/mL (± 8.4), with 28 and 27 cases of CRC, respectively (adjusted RR, 1.02; 95% CI, 0.60–1.74; P = 0.94) [77]. However, this study was limited by a small sample size (for CRC) and a short follow-up period. An RCT involving 36,282 post-menopausal females from 40 Women's Health Initiative (WHI) centers conducted from 1995–2005 provided participants with 1,000 mg of calcium carbonate and 400 IU of Vitamin D3 daily, or placebo; the average follow-up was 7.0 years (±1.4). The aim was to determine the potential preventative effects against CRC [78]. The incidence of CRC did not differ between the intervention and placebo groups (168 vs. 154 cases, respectively; HR, 1.08; 95% CI, 0.86–1.34; P = 0.51) [78]. However, this study had several limitations: the Vitamin D intake was too low to determine the significance of Vitamin D status between the groups (data regarding changes in serum 25(OH)D levels were not provided), there was poor treatment adherence, and the placebo group was allowed to take supplements outside of the study.

Post-hoc analysis of the 15,646 females (43%) who were not taking calcium or Vitamin D supplements outside of the study revealed that the risk of CRC in the intervention group fell by 17% when compared with that in the placebo group (HR, 0.83; 95% CI, 0.60–1.15; P = 0.27) [79]. A nationwide long-term RCT (VITAL) conducted from 2011–2017 in the US (25,871 participants [5,106 black]; age > 50 years) investigated the effects of Vitamin D on preventing invasive cancer and cardiovascular disease [80]. Participants were given Vitamin D3 of 2,000 IU/day or placebo over a median period of 5.3 years (3.8–6.1) [80]. The 40% increase in 25(OH)D levels (from 29.5 ng/mL to 41.8 ng/mL) after supplementation with Vitamin D did not result in a lower incidence of invasive cancer of any type (HR, 0.96; 95% CI, 0.88–1.06; P = 0.47), including CRC (51 cases vs. 47 cases; HR, 1.09; 95% CI, 0.73–1.62) compared with the placebo group; changes in 25(OH)D levels were insignificant [80].

Table 1. Summary of primary clinical trials examining Vitamin D supplementation and the incidence of CRC

First author (year)	Study name	No. of subjects	Age (yr)	Intervention (Vitamin D)	Duration (yr)	Mean serum 25(OH)D (ng/mL) at final	Objectives	No. of cases	HR	95% CI
Trivedi (2003) [77]	N/A	2,686 (males: 2,037 females: 649)	≥ 65	100,000 IU/4 mo	5	29.7 vs. 21.4	CRC incidence, CRC mortality	28 vs. 27 / 7 vs. 11	1.02 / 0.62	0.60–1.74, / 0.24–1.60
Wactawski-Wende (2006) [78]	WHI	36,282 (females only)	Post-menopausal	400 IU + 1,000 mg/d of Ca	7.0	N/A	CRC incidence	168 vs. 154	1.08	0.86–1.34
Baron (2015) [82]	N/A	2,259	45–75	1,000 IU/d	3 or 5	7.8 increasing in intervention group	CRA incidence	438 vs. 442	0.99	0.89–1.09
Lappe (2017) [81]	CAPS	2,303 (females only)	≥ 55	2,000 IU + 1,500 mg/d of Ca	4	42.5 vs. 30.9	All cancer mortality (CRC: 4 vs. 6)	45 vs. 64	0.70	0.48–1.02
Manson (2019) [80]	VITAL	25,871	≥ 50	2,000 IU /d	5.3	41.8 (40% increasing) vs. minimal change	CRC incidence	51 vs. 47	1.09	0.73–1.62

Ca, calcium; CRA, colorectal adenoma; CRC, colorectal cancer; HR, hazard ratio; CI, confidence interval; IU, international unit; WHI, Women's Health Initiative; VITAL, VITamin D and OmegA-3 TriaL.

A 4-year population-based RCT (CAPS) of 2,303 elderly females was conducted to determine whether supplementation with Vitamin D3 plus calcium reduced the risk of cancer among post-menopausal females [81]. The intervention group received 2,000 IU/d of Vitamin D3 and 1,500 mg/d of calcium; the mean 25(OH)D level (42.5 ng/mL [95% CI, 41.7–43.3]) in the intervention group did not result in a significantly lower risk of cancer of any type (3.89% vs. 5.58%; 95% CI, –0.06% to 3.46%; P = 0.06), including CRC (four cases vs. six cases), compared with the placebo group (30.9 ng/mL [95% CI, 30.2–31.6]) after 4 years [81]. A study similar to the VITAL and CAPS studies was conducted in Finland. The Finnish Vitamin D (FIND; NCT01463813) trial is a 5-year supplementation study of whether Vitamin D (D3 3,200 IU/d vs. 1,600 IU/d vs. placebo) prevented cardiovascular disease and cancer among 2,500 males and females aged 60+ years. The study has been completed and the results are awaited.

A study of whether Vitamin D prevents CRA examined 2,259 participants recently diagnosed with adenoma within the previous 4 months; patients were randomly assigned to receive daily calcium (1,200 mg) and Vitamin D3 (1,000 IU) or placebo A follow-up colonoscopy was performed 3 or 5 years after the index colonoscopy [82]. Overall, 43% of participants had metachronous adenoma at follow-up; neither Vitamin D alone (adjusted risk ratio, 0.99; 95% CI, 0.89–1.09) nor Vitamin D plus calcium (adjusted RR, 0.93; 95% CI, 0.80–1.08) showed significant efficacy for preventing colorectal polyps, regardless of the degree of change in serum 25(OH)D levels [82].

Thus, trials published to date show that Vitamin D supplementation does not prevent CRC or CRA (Table 1). However, the study by Trivedi had a small sample size, the WHI study used a dosage that was too low for significance and reported poor compliance, and the primary objective of the VITAL and CAPS trials was not the incidence of CRC. Therefore, further research is necessary to assess the possible roles of Vitamin D in preventing CRC.

Vitamin D intake and survival of CRC patients

Clinical trials assessing the benefits of Vitamin D supplementation in patients with CRC are warranted. An RCT by Trivedi et al. [77] showed that subgroup mortality was not different between the intervention and placebo groups (7/28 vs. 11/27; RR. 0.62, 95% CI, 0.24–1.60; P = 0.33). A phase 2 RCT (SUNSHINE) of 139 patients (44.1% male, 77.0% white race) with advanced or metastatic CRC was conducted from March 2012 through November 2016 to compare the effect of high (4,000 IU/d) versus standard (400 IU/d) dose Vitamin D3 supplementation plus standard chemotherapy; the median follow-up was 22.9 months [83]. Addition of high versus standard dose Vitamin D3 to standard chemotherapy resulted in a difference in median serum 25(OH)D levels (34.8 ng/mL vs. 18.7 ng/mL for standard chemotherapy; P < 0.001), but showed no significant difference in progression-free survival

(13.0 vs. 11.0 months for standard chemotherapy; log-rank P = 0.07) [83]. However, the multivariate HR (adjusted according to prognostic covariates for progression-free survival or death) was significant (HR, 0.64; one-sided 95% CI, 0–0.90; P = 0.02) [83].

The AMATERASU trial enrolled patients with gastrointestinal cancer (stages I to III) at a single center in Japan; the aim was to determine whether post-operative Vitamin D3 supplementation improves survival [84]. A total of 417 patients (48% of whom also had CRC) were assigned randomly to Vitamin D (2,000 IU/day) or placebo groups during a median follow-up of 3.5 years. The 5-year relapse-free survival was not different in overall gastrointestinal cancers (77% vs. 69%; HR, 0.76; 95% CI, 0.50–1.14; P = 0.18) and CRC (HR, 0.69; 95% CI, 0.39–1.24; P = 0.22), respectively [84]. A combined meta-analysis of the SUNSHINE and AMATERASU trials revealed that the HR for progression-free survival of CRC was 0.65 (0.36–0.94) [85].

CONCLUSION

The intestinal tract shows highest expression of VDR. In addition, calcitriol, the most active Vitamin D metabolite, has many important homeostatic actions in the intestine. Various Vitamin D signaling pathways may elicit chemopreventive and therapeutic effects against CRC. Epidemiological studies suggest that Vitamin D deficiency increases the incidence of CRC, and that it has a negative impact on survival of CRC patients. However, clinical trials assessing the benefits of Vitamin D supplementation in CRC are inconclusive; thus, further studies are needed to clarify the role of Vitamin D in CRC. Future studies should also examine potential genetic influences on the effects of Vitamin D supplementation (including Vitamin D metabolism, expression of the Vitamin D receptor and/or proteins that bind Vitamin D); such studies may help to identify patients most likely to benefit from supplements.

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REFERENCES

1. Quezada-Gutiérrez C, Álvarez-Bañuelos MT, Morales-Romero J, Sampieri CL, Guzmán-García RE, Montes-Villaseñor E. Factors associated with the survival of colorectal cancer in Mexico. Intest Res 2020;18:315-24.

2. Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol 2021;14:101174.

3. Gweon TG, Yoon KT, Kim CH, Kim JJ. Postgastrectomy gastric cancer patients are at high risk for colorectal neoplasia: a case control study. Intest Res 2021;19:239-46.

4. Kim SH, Lim YJ. The role of microbiome in colorectal carcinogenesis and its clinical potential as a target for cancer treatment. Intest Res 2022;20:31-42.

5. Valiati B, Perez RO, Kotze PG. Education levels and survival in colorectal cancer: is there really an obvious association? Intest Res 2020;18:247-8.

6. Choi YS, Kim WS, Hwang SW, Park SH, Yang DH, Ye BD, et al. Clinical outcomes of submucosal colorectal cancer diagnosed after endoscopic resection: a focus on the need for surgery. Intest Res 2020;18:96-106.

7. Murphy N, Moreno V, Hughes DJ, Vodicka L, Vodicka P, Aglago EK, et al. Lifestyle and dietary environmental factors in colorectal cancer susceptibility. Mol Aspects Med 2019;69:2-9.

8. Ferrer-Mayorga G, Larriba MJ, Crespo P, Muñoz A. Mechanisms of action of vitamin D in colon cancer. J Steroid Biochem Mol Biol 2019;185:1-6.

9. Alfredsson L, Armstrong BK, Butterfield DA, Chowdhury R, de Gruijl FR, Feelisch M, et al. Insufficient sun exposure has become a real public health problem. Int J Environ Res Public Health 2020;17:5014.

10. Deeb KK, Trump DL, Johnson CS. Vitamin D signalling pathways in cancer: potential for anticancer therapeutics. Nat Rev Cancer 2007;7:684-700.

11. Dobson R, Cock HR, Brex P, Giovannoni G. Vitamin D supplementation. Pract Neurol 2018;18:35-42.

12. Al-Ghafari AB, Balamash KS, Al Doghaither HA. Relationship between serum vitamin D and calcium levels and vitamin D receptor gene polymorphisms in colorectal cancer. Biomed Res Int 2019;2019:8571541.

13. Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. Mayo Clin Proc 2010;85:752-7; quiz 757-8.

14. Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. Rev Endocr Metab Disord 2017;18:153-65.

15. Daly RM, Gagnon C, Lu ZX, Magliano DJ, Dunstan DW, Sikaris KA, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. Clin Endocrinol (Oxf) 2012;77:26-35.

16. Crooks B, Misra R, Arebi N, Kok K, Brookes MJ, McLaughlin J, et al. The dietary practices and beliefs of British South Asian people living with inflammatory bowel disease: a multicenter study from the United Kingdom. Intest Res 2022;20:53-63.

17. Shafiee NH, Manaf ZA, Mokhtar NM, Raja Ali RA. Anti-inflammatory diet and inflammatory bowel disease: what clinicians and patients should know? Intest Res 2021;19:171-85.

18. Hu N, Zhang H. CYP24A1 depletion facilitates the antitumor effect of vitamin D3 on thyroid cancer cells. Exp Ther Med 2018;16:2821-30.

19. Kósa JP, Horváth P, Wölfling J, Kovács D, Balla B, Mátyus P, et al. CYP24A1 inhibition facilitates the anti-tumor effect of vitamin D3 on colorectal cancer cells. World J Gastroenterol 2013;19:2621-8.

20. Wang P, Qin X, Liu M, Wang X. The burgeoning role of cytochrome P450-mediated vitamin D metabolites against colorectal cancer. Pharmacol Res 2018;133:9-20.

21. Lucas RM, Gorman S, Geldenhuys S, Hart PH. Vitamin D and immunity. F1000Prime Rep 2014;6:118.

22. Serrano D, Pozzi C, Guglietta S, Fosso B, Suppa M, Gnagnarella P, et al. Microbiome as mediator of diet on colorectal cancer risk: the role of vitamin D, markers of inflammation and adipokines. Nutrients 2021;13:363.

23. Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. Nat Rev Gastroenterol Hepatol 2019;16:690-704.

24. Zhou X, Chen C, Zhong YN, Zhao F, Hao Z, Xu Y, et al. Effect and mechanism of vitamin D on the development of colorectal cancer based on intestinal flora disorder. J Gastroenterol Hepatol 2020;35:1023-31.

25. Bellerba F, Muzio V, Gnagnarella P, Facciotti F, Chiocca S, Bossi P, et al. The association between vitamin D and gut microbiota: a systematic review of human studies. Nutrients 2021;13:3378.

26. Muñoz A, Grant WB. Vitamin D and cancer: an historical overview of the epidemiology and mechanisms. Nutrients 2022; 14:1448.

27. Pálmer HG, González-Sancho JM, Espada J, Berciano MT, Puig I, Baulida J, et al. Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. J Cell Biol 2001;154:369-87.

28. Lamprecht SA, Lipkin M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nat Rev Cancer 2003;3:601-14.

29. Klampfer L. Vitamin D and colon cancer. World J Gastrointest Oncol 2014;6:430-7.

30. Chen A, Davis BH, Sitrin MD, Brasitus TA, Bissonnette M. Transforming growth factor-beta 1 signaling contributes to Caco-2 cell growth inhibition induced by 1,25(OH)(2)D(3). Am J Physiol Gastrointest Liver Physiol 2002;283:G864-74.

31. Fedirko V, Mandle HB, Zhu W, Hughes DJ, Siddiq A, Ferrari P, et al. Vitamin D-related genes, blood vitamin D levels and colorectal cancer risk in Western European populations. Nutrients 2019; 11:1954.

32. van Harten-Gerritsen AS, Balvers MG, Witkamp RF, Kampman E, van Duijnhoven FJ. Vitamin D, inflammation, and colorectal cancer progression: a review of mechanistic studies and future directions for epidemiological studies. Cancer Epidemiol Biomarkers Prev 2015;24:1820-8.

33. Scaglione-Sewell BA, Bissonnette M, Skarosi S, Abraham C, Brasitus TA. A vitamin D3 analog induces a G1-phase arrest in CaCo-2 cells by inhibiting cdk2 and cdk6: roles of cyclin E, p21Waf1, and p27Kip1. Endocrinology 2000;141:3931-9.

34. Beildeck ME, Islam M, Shah S, Welsh J, Byers SW. Control of TCF-4 expression by VDR and vitamin D in the mouse mammary gland and colorectal cancer cell lines. PLoS One 2009;4:e7872.

35. Razak S, Afsar T, Almajwal A, Alam I, Jahan S. Growth inhibition and apoptosis in colorectal cancer cells induced by Vitamin D-Nanoemulsion (NVD): involvement of Wnt/β-catenin and other signal transduction pathways. Cell Biosci 2019;9:15.

36. Byers SW, Rowlands T, Beildeck M, Bong YS. Mechanism of action of vitamin D and the vitamin D receptor in colorectal cancer prevention and treatment. Rev Endocr Metab Disord 2012;13:31-8.

37. Javed M, Althwanay A, Ahsan F, Oliveri F, Goud HK, Mehkari Z, et al. Role of vitamin D in colorectal cancer: a holistic approach and review of the clinical utility. Cureus 2020;12:e10734.

38. Kure S, Nosho K, Baba Y, Irahara N, Shima K, Ng K, et al. Vitamin D receptor expression is associated with PIK3CA and KRAS mutations in colorectal cancer. Cancer Epidemiol Biomarkers Prev 2009;18:2765-72.

39. El-Sharkawy A, Malki A. Vitamin D signaling in inflammation and cancer: molecular mechanisms and therapeutic implications. Molecules 2020;25:3219.

40. Li YC, Chen Y, Du J. Critical roles of intestinal epithelial vitamin D receptor signaling in controlling gut mucosal inflammation. J Steroid Biochem Mol Biol 2015;148:179-83.

41. Song M, Garrett WS, Chan AT. Nutrients, foods, and colorectal cancer prevention. Gastroenterology 2015;148:1244-60.e16.

42. Rawson JB, Sun Z, Dicks E, Daftary D, Parfrey PS, Green RC, et al. Vitamin D intake is negatively associated with promoter methylation of the Wnt antagonist gene DKK1 in a large group of colorectal cancer patients. Nutr Cancer 2012;64:919-28.

43. Zuo S, Wu L, Wang Y, Yuan X. Long non-coding RNA MEG3 activated by vitamin D suppresses glycolysis in colorectal cancer via promoting c-Myc degradation. Front Oncol 2020;10:274.

44. Song M, Lee IM, Manson JE, Buring JE, Dushkes R, Gordon D, et al. No association between vitamin D supplementation and risk of colorectal adenomas or serrated polyps in a randomized trial. Clin Gastroenterol Hepatol 2021;19:128-35.e6.

45. Wesselink E, Kok DE, Bours MJL, de Wilt JHW, van Baar H, van Zutphen M, et al. Vitamin D, magnesium, calcium, and their interaction in relation to colorectal cancer recurrence and all-cause mortality. Am J Clin Nutr 2020;111:1007-17.

46. Shang J, Zhu Z, Chen Y, Song J, Huang Y, Song K, et al. Small-molecule activating SIRT6 elicits therapeutic effects and synergistically promotes anti-tumor activity of vitamin D$_3$ in colorectal cancer. Theranostics 2020;10:5845-64.

47. Boughanem H, Cabrera-Mulero A, Hernández-Alonso P, Clemente-Postigo M, Casanueva FF, Tinahones FJ, et al. Association between variation of circulating 25-OH vitamin D and methylation of secreted frizzled-related protein 2 in colorectal cancer. Clin Epigenetics 2020;12:83.

48. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol 1980;9:227-31.

49. Ross AC, Taylor CL, Yaktine AL, Del Valle HB. Dietary reference intakes for calcium and vitamin D. Washington DC, National Academies Press, 2011.

50. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30. Erratum in: J Clin Endocrinol Metab 2011;96:3908.

51. Ng K, Sargent DJ, Goldberg RM, Meyerhardt JA, Green EM, Pitot HC, et al. Vitamin D status in patients with stage IV colorectal cancer: findings from Intergroup trial N9741. J Clin Oncol 2011;29:1599-606.

52. Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. Bone 2002;30:771-7.

53. van Dam RM, Snijder MB, Dekker JM, Stehouwer CD, Bouter LM, Heine RJ, et al. Potentially modifiable determinants of vitamin D status in an older population in the Netherlands: the Hoorn Study. Am J Clin Nutr 2007;85:755-61.

54. Yuan C, Sato K, Hollis BW, Zhang S, Niedzwiecki D, Ou FS, et al. Plasma 25-hydroxyvitamin D levels and survival in patients with advanced or metastatic colorectal cancer: findings from CALGB/SWOG 80405 (Alliance). Clin Cancer Res 2019;25:7497-505.

55. Chandler PD, Buring JE, Manson JE, Giovannucci EL, Moorthy MV, Zhang S, et al. Circulating vitamin D levels and risk of colorectal cancer in women. Cancer Prev Res (Phila) 2015;8:675-82.

56. McCullough ML, Zoltick ES, Weinstein SJ, Fedirko V, Wang M, Cook NR, et al. Circulating vitamin D and colorectal cancer risk: an international pooling project of 17 cohorts. J Natl Cancer Inst 2019;111:158-69.

57. Huang D, Lei S, Wu Y, Weng M, Zhou Y, Xu J, et al. Additively protective effects of vitamin D and calcium against colorectal adenoma incidence, malignant transformation and progression: a systematic review and meta-analysis. Clin Nutr 2020;39:2525-38.

58. Zhang L, Zou H, Zhao Y, Hu C, Atanda A, Qin X, et al. Association between blood circulating vitamin D and colorectal cancer risk in Asian countries: a systematic review and dose-response meta-analysis. BMJ Open 2019;9:e030513.

59. Wei MY, Garland CF, Gorham ED, Mohr SB, Giovannucci E. Vitamin D and prevention of colorectal adenoma: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2008;17:2958-69.

60. Fedirko V, Bostick RM, Goodman M, Flanders WD, Gross MD. Blood 25-hydroxyvitamin D3 concentrations and incident sporadic colorectal adenoma risk: a pooled case-control study. Am J Epidemiol 2010;172:489-500.

61. Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: serum vitamin D and colorectal adenoma risk. Prev Med 2011;53:10-6.

62. Choi YJ, Kim YH, Cho CH, Kim SH, Lee JE. Circulating levels of vitamin D and colorectal adenoma: a case-control study and a meta-analysis. World J Gastroenterol 2015;21:8868-77.

63. Maalmi H, Ordóñez-Mena JM, Schöttker B, Brenner H. Serum 25-hydroxyvitamin D levels and survival in colorectal and breast cancer patients: systematic review and meta-analysis of prospective cohort studies. Eur J Cancer 2014;50:1510-21.

64. Ng K, Meyerhardt JA, Wu K, Feskanich D, Hollis BW, Giovannucci EL, et al. Circulating 25-hydroxyvitamin d levels and survival in patients with colorectal cancer. J Clin Oncol 2008; 26:2984-91.

65. Mezawa H, Sugiura T, Watanabe M, Norizoe C, Takahashi D, Shimojima A, et al. Serum vitamin D levels and survival of patients with colorectal cancer: post-hoc analysis of a prospective cohort study. BMC Cancer 2010;10:347.

66. Fedirko V, Riboli E, Tjønneland A, Ferrari P, Olsen A, Bueno-de-Mesquita HB, et al. Prediagnostic 25-hydroxyvitamin D, VDR and CASR polymorphisms, and survival in patients with colorectal cancer in western European ppulations. Cancer Epidemiol Biomarkers Prev 2012;21:582-93.

67. Tretli S, Schwartz GG, Torjesen PA, Robsahm TE. Serum levels of 25-hydroxyvitamin D and survival in Norwegian patients with cancer of breast, colon, lung, and lymphoma: a population-based study. Cancer Causes Control 2012;23:363-70.

68. Wu G, Xue M, Zhao Y, Han Y, Zhang S, Zhang J, et al. Low circulating 25-hydroxyvitamin D level is associated with increased colorectal cancer mortality: a systematic review and dose-response meta-analysis. Biosci Rep 2020;40:BSR20201008.

69. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. J Clin Endocrinol Metab 2004;89:5387-91.

70. Trang HM, Cole DE, Rubin LA, Pierratos A, Siu S, Vieth R. Evidence that vitamin D3 increases serum 25-hydroxyvitamin D more efficiently than does vitamin D2. Am J Clin Nutr 1998;68:854-8.

71. Houghton LA, Vieth R. The case against ergocalciferol (vitamin D2) as a vitamin supplement. Am J Clin Nutr 2006;84:694-7.

72. Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev 2014; (1):CD007470.

73. Wacker M, Holick MF. Vitamin D - effects on skeletal and extraskeletal health and the need for supplementation. Nutrients 2013;5:111-48.

74. Berkovich L, Sintov AC, Ben-Shabat S. Inhibition of cancer growth and induction of apoptosis by BGP-13 and BGP-15, new calcipotriene-derived vitamin D3 analogs, in-vitro and in-vivo studies. Invest New Drugs 2013;31:247-55. Erratum in: Invest New Drugs 2013;31:490.

75. Milczarek M, Psurski M, Kutner A, Wietrzyk J. Vitamin D analogs enhance the anticancer activity of 5-fluorouracil in an in vivo mouse colon cancer model. BMC Cancer 2013;13:294.

76. Milczarek M, Rosinska S, Psurski M, Maciejewska M, Kutner A, Wietrzyk J. Combined colonic cancer treatment with vitamin D analogs and irinotecan or oxaliplatin. Anticancer Res 2013;33:433-44.

77. Trivedi DP, Doll R, Khaw KT. Effect of four monthly oral vitamin D3 (cholecalciferol) supplementation on fractures and mortality in men and women living in the community: randomised double blind controlled trial. BMJ 2003;326:469.

78. Wactawski-Wende J, Kotchen JM, Anderson GL, Assaf AR, Brunner RL, O'Sullivan MJ, et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Engl J Med 2006;354:684-96. Erratum in: N Engl J Med 2006;354:1102.

79. Bolland MJ, Grey A, Gamble GD, Reid IR. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. Am J Clin Nutr 2011;94:1144-9.

80. Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. N Engl J Med 2019;380:33-44.

81. Lappe J, Watson P, Travers-Gustafson D, Recker R, Garland C, Gorham E, et al. Effect of vitamin D and calcium supplementation on cancer incidence in older women: a randomized clinical trial. JAMA 2017;317:1234-43.

82. Baron JA, Barry EL, Mott LA, Rees JR, Sandler RS, Snover DC, et al. A trial of calcium and vitamin D for the prevention of colorectal adenomas. N Engl J Med 2015;373:1519-30.

83. Ng K, Nimeiri HS, McCleary NJ, Abrams TA, Yurgelun MB, Cleary JM, et al. Effect of high-dose vs standard-dose vitamin D3 supplementation on progression-free survival among patients with advanced or metastatic colorectal cancer: the SUNSHINE randomized clinical trial. JAMA 2019;321:1370-9.

84. Urashima M, Ohdaira H, Akutsu T, Okada S, Yoshida M, Kitajima M, et al. Effect of vitamin D supplementation on relapse-free survival among patients with digestive tract cancers: the AMATERASU randomized clinical trial. JAMA 2019;321:1361-9.

85. Vaughan-Shaw PG, Buijs LF, Blackmur JP, Theodoratou E, Zgaga L, Din FVN, et al. The effect of vitamin D supplementation on survival in patients with colorectal cancer: systematic review and meta-analysis of randomised controlled trials. Br J Cancer 2020;123:1705-12.