

Review

# Vitamin D and Colorectal, Breast, and Prostate Cancers: A Review of the Epidemiological Evidence

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## Abstract

Over the past two decades, the question of whether vitamin D has a role in cancer incidence, progression, and mortality has been studied in detail. Colorectal, breast, and prostate cancers have been a particular area of focus; together, these three malignancies account for approximately 35% of cancer cases and 20% of cancer deaths in the United States, and as such are a major public health concern. Herein, we review and synthesize the epidemiological research regarding vitamin D, as measured by the biomarker 25-hydroxycholecalciferol [25(OH)D], and the incidence, progression, and mortality of these cancers. Overall, the results of observational studies of the relationship between 25(OH)D and colorectal cancer have revealed a consistent inverse association for incidence and mortality; while for breast cancer, results have generally demonstrated a relationship between higher 25(OH)D and lower risk for progression and mortality. In contrast, randomized, double-blind clinical trials conducted to date have generally failed to support these findings. For prostate cancer, there is no convincing evidence of an association between 25(OH)D and incidence, and inconsistent data for progression and mortality, though results of one open label clinical trial suggest that supplementation with 4000 IU/d of vitamin D<sub>3</sub> may inhibit progression of the disease. Nonetheless, until the results of additional ongoing randomized, double-blind clinical trials are reported, it will be difficult to ascertain if vitamin D itself is related to a reduction in risk for some cancer endpoints, or whether high concentrations of the vitamin D biomarker 25(OH)D may instead serve as a marker for an overall beneficial risk factor profile.

Key words: Vitamin D, colorectal cancer, breast cancer, prostate cancer, 25(OH)D

## Introduction

Vitamin D has been the subject of intense scrutiny in relation to various cancer endpoints, with particular focus on the incidence and mortality of colorectal, breast, and prostate cancers. Together, these three malignancies account for approximately 35% of cancer cases and 20% of cancer deaths in the United States<sup>1</sup>. After more than two decades of comprehensive efforts to elucidate the role of vitamin D in cancer, we now have the opportunity to synthesize the cumulative knowledge about this subject in order to evaluate whether vitamin D is related either positively or negatively to cancer incidence, prognosis,

and/or survival in relation to colorectal, breast, and prostate cancers.

Conducting and interpreting epidemiological studies of vitamin D and health outcomes can be complex due to inherent nature of the biomarkers employed to evaluate vitamin D status. First, dietary studies of vitamin D are somewhat limited because of the major contribution of endogenous synthesis after UV exposure to circulating concentrations of the vitamin D metabolite 25-hydroxycholecalciferol [25(OH)D]. In part because it captures both dietary intake and endogenous synthesis of vitamin D, total

circulating concentration of 25(OH)D is the biomarker that is most frequently used in epidemiological investigations; for this reason, the present review will include only those investigations that employed 25(OH)D as a marker of vitamin D status. In contrast, the active vitamin D metabolite 1,25-dihydroxycholecalciferol [1,25(OH)<sub>2</sub>D], which is produced after hydroxylation of 25(OH)D, is the primary metabolite employed for *in vitro* experiments, which have provided extensive evidence of several potential mechanisms of action for vitamin D in carcinogenesis. There are general limitations to the use of either biomarker. In the case of 1,25(OH)<sub>2</sub>D, it is subject to tight homeostatic regulation and as such does not vary as greatly in human populations as does 25(OH)D. On the other hand, circulating 25(OH)D is influenced by an array of individual characteristics that are themselves related to cancer risk either directly or indirectly, including diet, body size, physical activity, sun exposure, and skin pigmentation. We will revisit the implications of this epidemiological challenge later in the review in the context of the work conducted to date regarding colorectal, breast, and prostate cancer incidence and mortality.

## Colorectal Cancer

An estimated 93,090 new cases of colon cancer and 39,610 cases of rectal cancer are anticipated in 2015<sup>1</sup>. Among men and women combined, colorectal malignancies are the second most common cause of cancer mortality in the United States, with approximately 50,000 deaths each year<sup>2</sup>. The primary precursor lesions for colorectal cancer are adenomas<sup>3</sup>, and a meta-analysis of the presence of adenomas among U.S. adults has estimated a range from 22% to over 50%, with a pooled prevalence of approximately 30%<sup>4</sup>. Among individuals in whom an adenoma has been detected and removed, 10-15% per year will go on to develop another, recurrent, adenoma<sup>5</sup>. Thus, examining the role of vitamin D in colorectal adenoma incidence and recurrence provides important information regarding its potential for preventing colorectal malignancies during the first steps in the carcinogenesis pathway.

To date, meta-analyses of the association between serum 25(OH)D and colorectal adenoma have consistently demonstrated a statistically significant inverse relationship for incidence, but not recurrence, though data for the latter outcome are comparatively sparse<sup>6,7</sup>. Since the results of these meta-analyses were reported, a third study of adenoma recurrence was published, which again showed no statistically significant association between 25(OH)D and odds of adenoma recurrence<sup>8</sup>. Underlying mechanisms for the observed differences in the association for 25(OH)D

by incident vs. recurrent adenomas are currently only speculative. Differences in methylation patterns during adenoma growth and development<sup>9</sup> and variation in expression of key vitamin D pathway enzymes such as CYP24A1 in adenoma tissue during different stages of adenoma development<sup>10,11</sup> are two potential pathways through which vitamin D may exert differential effects on adenoma incidence vs. recurrence. It is also possible that individuals included in studies of recurrent lesions represent a population of "polyp formers" for whom the risk factor profile is different, and/or for whom the carcinogenic pathway is not affected by vitamin D. Taken together, these observational studies indicate that vitamin D may have a role in reducing the risk of incident colorectal adenomas, but after removal of these lesions, there is no evidence that it will prevent the formation of another. Results of the Vitamin D/Calcium Polyp Prevention Study<sup>12</sup>, a large, double-blind, randomized clinical trial of vitamin D and calcium supplementation for the prevention of colorectal adenoma recurrence, are pending and are expected to provide more definitive data. We will next move forward in the carcinogenesis pathway to consider the potential role of vitamin D in the incidence of colorectal cancer.

To date, several meta-analyses of blood 25(OH)D concentrations and colorectal cancer incidence have been conducted, and all have shown a statistically significant inverse association<sup>13-16</sup>. In contrast to the results for breast cancer, as discussed further below, the majority of studies conducted to assess the relationship between 25(OH)D and colorectal cancer incidence are prospective investigations that have reported a significantly reduced risk for colorectal cancer with higher 25(OH)D concentrations. When comparing the highest vs. lowest categories of 25(OH)D levels, consistent estimates of 0.67 (0.54-0.80)<sup>13</sup> and 0.66 (0.54-0.81)<sup>16</sup> were yielded for colorectal cancer risk. In contrast, for analogous analyses employing the endpoint of colorectal adenoma, the magnitude of effect has tended to be somewhat weaker and less consistent<sup>6,7</sup>. Further, as described above, 25(OH)D levels have generally not been found to prevent recurrent lesions. As hypothesized by Yin et al.<sup>6</sup>, these findings support the concept that vitamin D may not inhibit the formation of new adenomas, but rather that it may have a role in inhibiting growth of existing lesions and/or progression through the carcinogenesis pathway.

When examining observational studies in greater detail, further information about potential sub-site and sex-specific effects emerges. Some studies have indicated that the association with 25(OH)D is stronger in rectal cancers than in colon cancers<sup>17,18</sup>; however, others have reported a potentially stronger

effect for colon cancers than rectal or colorectal cancers<sup>19,20</sup>. In one of the two studies where a stronger effect for rectal cancer was observed, women were included in the study population<sup>18</sup>. In the two studies for which a stronger association with colon cancer was demonstrated, both included men and women<sup>19,20</sup>. The paucity of sub-site specific data for each sex separately, as well as the often-limited number of cases available for analysis by sub-site, precludes drawing firm conclusions about whether vitamin D may have differential effects in men vs. women with regard to colorectal cancer incidence. However, it is worth noting that in study populations that included only women, a significant inverse association between 25(OH)D and colorectal cancer overall was observed<sup>21,22</sup>; while for studies including only men, the results were either null, showed a stronger association for rectal rather than colon cancer, or demonstrated a direct relationship between 25(OH)D and colorectal cancer whereby higher concentrations were related to an increased risk for colorectal cancer<sup>17,23,24</sup>. The results from observational studies suggest the possibility that women may experience a greater benefit from higher 25(OH)D levels in relation to the development of colorectal cancer overall; however, despite intensive research, firm conclusions cannot be drawn for a sex-specific effect. In summary, while there is a consistent association between 25(OH)D and colorectal cancer incidence reported in observational epidemiological studies, colorectal sub-site- or sex-specific effects cannot be ascertained from the current literature.

Randomized, controlled clinical trials provide the best evidence for whether vitamin D might reduce the risk of colorectal cancer, and to date, only two have been published with data specific to colorectal cancer<sup>22,25</sup>. The Women's Health Initiative (WHI) randomized women to 400 IU vitamin D<sub>3</sub> and 1000 mg of calcium vs. placebo. No differences in risk for colorectal cancer by treatment group were observed, although some limitations of the trial were noted, including the relatively healthy study population, the timing of the intervention, and the comparatively short follow-up time<sup>22</sup>. In addition, it has been suggested that the dose of vitamin D used in WHI was too low to elicit protective effects<sup>26</sup>. Another clinical trial conducted in the United Kingdom randomized men and women to receive 100,000 IU/d of vitamin D<sub>3</sub> every four months for five years<sup>25</sup>. No reduction in risk of either colorectal cancer incidence or mortality was observed<sup>25</sup>. Thus, while association studies of 25(OH)D and colorectal cancer incidence indicate a potential risk reduction with higher concentrations of this vitamin D biomarker, evidence from randomized clinical trials does not support this finding. Results

from the ongoing Vitamin D and Omega-3 Trial (VITAL) trial, in which participants are supplemented with 2000 IU/d of vitamin D<sub>3</sub> with and without an omega-3 fatty acid supplement to ascertain whether the intervention can prevent the development of cancer or cardiovascular disease<sup>27</sup>, will likely provide more definitive evidence regarding whether vitamin D is a viable strategy for colorectal cancer prevention. Next, we will explore the studies of 25(OH)D and colorectal cancer progression and survival.

Two investigations have assessed the association between 25(OH)D concentrations and colorectal cancer progression specifically. Mezawa et al.<sup>28</sup> measured blood levels of 25(OH)D in Stage I-IV patients at the time of surgery for this malignancy and found that although 25(OH)D levels were significantly directly related to overall survival, they were not associated with disease-free survival. In another study conducted among Stage IV colorectal cancer patients undergoing chemotherapy, concentrations of 25(OH)D were not significantly associated with time to progression of disease<sup>29</sup>. Therefore, to date, there is no evidence that vitamin D is associated with inhibiting colorectal cancer progression *per se*, although it may have an impact on cancer-related mortality.

Several prospective epidemiological investigations have been completed that were designed to ascertain if there is a relationship between 25(OH)D and deaths associated with colorectal cancer and/or all-cause mortality<sup>28-35</sup>, with equivocal results. Five studies have reported a significant inverse association between 25(OH)D and either colorectal cancer-specific or all-cause mortality<sup>28,31,33-35</sup>, with three others observing null relationships<sup>29,30,32</sup>. The reasons for the differential results between these studies are unclear; there did not appear to be general marked variation in the stage at diagnosis, sex, or study design (pre- vs. post-diagnostic samples) between the studies that showed an association as compared to those that did not. Three of the five studies demonstrating a statistically significant outcome were conducted outside of the United States; one from the Study of Colorectal Cancer in Scotland (SOCCS)<sup>33</sup>; one from the European Prospective Investigation into Cancer and Nutrition (EPIC), which included participants from Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom<sup>31</sup>; and one from Japan<sup>28</sup>. However, given the variation in latitude and diet between the participant counties, it is difficult to draw firm conclusions regarding the potential relationship between 25(OH)D and survival. For this, we must consider several meta-analyses<sup>36-38</sup> that have recently been completed. Each has reported that higher concentrations of 25(OH)D were significantly inversely related to re-

duced risk for cancer-specific and/or overall mortality among patients with colorectal cancer. Based on these findings, there is consistent evidence of an inverse association between 25(OH)D and colorectal cancer-related mortality. However, as discussed further below, caution is warranted in drawing conclusions about causality from these studies.

## Breast Cancer

It has been estimated that there will be 231,840 new cases of female breast cancer and 40,290 deaths from this disease in 2015<sup>1</sup>. Unlike with colorectal carcinogenesis, there are limited data regarding vitamin D and breast cancer precursor lesions. In one study among participants in the WHI, Rohan et al. found no reduction in risk for benign proliferative breast disease, a condition associated with increased risk for breast cancer (HR=0.99, 95% CI=0.86-1.13), among women receiving calcium and vitamin D<sup>39</sup>. In contrast to studies of breast precursor lesions, there are a number of reports regarding vitamin D and breast cancer incidence, progression, and mortality.

Four thorough meta-analyses that included both case-control and prospective studies of the relationship between circulating concentrations of 25(OH)D and risk for breast cancer have been published to date<sup>14,40-42</sup>. When presenting the point estimates for both types of epidemiological studies combined, results have generally shown a statistically significant inverse relationship between 25(OH)D and breast cancer<sup>14,40,41</sup>. However, several groups that have examined the results by study design reported a marked and important difference in results depending on the type of epidemiological study<sup>14,40,43</sup>. Specifically, case-control studies have generally shown a statistically significant reduction in risk for breast cancer associated with higher circulating concentrations of 25(OH)D, with reported summary ORs (95% CIs) from separate meta-analyses of 0.59 (0.48, 0.73)<sup>40</sup> and 0.83 (0.79, 0.87)<sup>14</sup> for the highest vs. lowest category of 25(OH)D levels. In contrast, prospective studies have shown no significant relationship between 25(OH)D and breast cancer incidence, with summary statistics of 0.92 (0.82, 1.04)<sup>40</sup> and 0.97 (0.92, 1.03)<sup>14</sup>. Further, since the publication of these meta-analyses, numerous additional reports have been published<sup>41,42,44-57</sup>. These studies have maintained a striking fidelity with the general pattern of case-control studies reporting a significant association and prospective findings being null. This distinction is critical to the interpretation of the overall body of literature related to vitamin D and cancer.

As mentioned previously, concentrations of 25(OH)D are influenced not only by dietary intake, but also by important risk factors for cancer such as

body size and sun exposure, both of which are associated with a third cancer-related variable, physical activity. It has been clearly demonstrated that concentrations of 25(OH)D are lower among those with higher body mass index and lower physical activity levels, which themselves are documented outcomes after a diagnosis of breast cancer<sup>58,59</sup>. We and others<sup>14,59,60</sup> have suggested that case-control studies, with blood from cases being drawn after diagnosis, have substantial limitations due to the potential influence of a breast cancer diagnosis on 25(OH)D concentrations, rather than the converse. Further, the most recent meta-analysis of all studies of 25(OH)D and breast cancer, which included the majority of work through 2013, reported a null association for measured 25(OH)D and incidence, with a pooled OR (95% CI) of 0.92 (95% CI: 0.83-1.02) for the highest category of 25(OH)D compared to the lowest<sup>42</sup>, regardless of study design. To date, the only large randomized clinical trial that has been completed is the WHI, which demonstrated no reduction in risk for breast cancer among women receiving 400 IU vitamin D<sub>3</sub> and 1000 mg of calcium vs. placebo<sup>61</sup>. In total, the evidence does not support a role for vitamin D in the prevention of breast cancer incidence. The literature regarding vitamin D and breast cancer prognosis and survival will next be considered.

Two meta-analyses of the association between vitamin D and breast cancer survival have recently been published, and both reported that higher concentrations of 25(OH)D were related to better survival among women diagnosed with breast cancer<sup>42,62</sup>. These findings clearly reflect the body of literature on this subject, for which the majority of studies have reported a statistically significant relationship between higher blood 25(OH)D levels and less-advanced cancers or improved prognosis, including reduced risk for recurrence and increased breast cancer survival or all-cause survival<sup>32,63-66</sup>. In addition, in a retrospective review of patients by Zeichner et al.<sup>67</sup>, HER2<sup>+</sup> patients who were undergoing chemotherapy with trastuzumab and who also received vitamin D supplements experienced statistically significantly improved disease-free survival as compared to those on the same therapy who did not take a vitamin D supplement. In this study, the mean dose of vitamin D received by patients was 10,472 IU/week, or <1500 IU/d<sup>67</sup>. Taken together, the results for prognosis and survival generally provide a more consistent picture than for vitamin D and breast cancer incidence. These were well-conducted observational studies that generally controlled for factors that may confound the relationship between 25(OH)D and breast cancer survival such as body size, physical activity, and cancer stage at diagnosis. Nonetheless the

potential for residual confounding remains, particularly in light of the differential findings for vitamin D and breast cancer incidence by study design, and can be resolved only through the conduct of a randomized, double-blind, placebo-controlled trial of vitamin D supplementation among breast cancer patients.

## Prostate Cancer

With 220,800 cases and 27,540 deaths anticipated in 2015, prostate cancer remains a major public health challenge<sup>1</sup>. As mentioned above, increased skin pigmentation is associated with substantially reduced circulating concentrations of 25(OH)D, and as such, African-Americans have consistently been shown to have significantly lower 25(OH)D levels than any other group in the U.S.<sup>68,69</sup>. In part because African-American men also suffer from the highest incidence and mortality rates from prostate cancer<sup>1</sup>, it was originally hypothesized that both this health disparity and overall risk for prostate cancer in general might be at least partly attributable to vitamin D insufficiency<sup>70</sup>.

As with breast cancer, there are limited data regarding vitamin D and precursor lesions for prostate cancer. Gee et al.<sup>71</sup> administered either placebo (n=15) or 10 µg/d of 1α-hydroxyvitamin D<sub>2</sub> (n=16), a vitamin D analogue with low calcemic activity, to participants with high-grade prostatic intraepithelial neoplasia (HGPIN) prior to prostatectomy. After 28 days, no significant differences were observed between the two treatment groups for Gleason grade, proliferation, apoptosis, or angiogenesis, though a statistically significant difference was detected for plasma TGF-β2 levels, with lower levels observed among those who received 1α-hydroxyvitamin D<sub>2</sub><sup>71</sup>.

Regarding prostate cancer, approximately 30 studies of the association between 25(OH)D and prostate cancer incidence have been conducted<sup>24,53,55,72-95</sup>. Of these, only two have reported a clear and statistically significant inverse relationship for overall risk of prostate cancer<sup>75,80</sup>; while an additional five studies demonstrated an increased risk for prostate cancer associated with higher concentrations of 25(OH)D<sup>24,76,83,88,92</sup>. The remainder of the published reports are null for the endpoint of overall prostate cancer incidence; however, a meta-analysis published in 2014 of 21 studies reported a statistically significant finding that higher 25(OH)D concentrations were related to a higher risk for developing prostate cancer<sup>96</sup>. Taken together, epidemiological association studies have provided no convincing evidence that higher concentrations of 25(OH)D might reduce the risk of developing prostate cancer. In fact, the evidence indicating that higher 25(OH)D levels may be directly associated with an increased risk of prostate cancer is

stronger than for any putative protective relationship. Next, whether vitamin D might have an impact on progression of prostate cancer or prostate cancer mortality will be explored.

To date, several studies have reported a significant inverse association between 25(OH)D and more advanced prostate cancers<sup>89,91,94</sup>, which might suggest that vitamin D has an inhibitory effect on prostate cancer progression and/or that it may be critical in the carcinogenesis pathway from which clinically relevant, but not less aggressive, cancers arise<sup>97</sup>. However, other investigations have failed to show such a relationship<sup>82,85,95</sup>, or have even demonstrated a significantly increased risk for aggressive prostate cancer with higher levels of 25(OH)D<sup>83,84,88</sup>. Thus far only one clinical trial has been completed which provides evidence of a role for vitamin D in prostate cancer progression. In this open-label study, a total of 44 men with low-risk prostate cancer completed a trial in which they were administered 4000 IU/d of vitamin D<sub>3</sub> for one year in order to determine whether the supplement could inhibit cancer progression or reduce prostate-specific antigen levels as compared to 19 historical controls<sup>98</sup>. No marked differences in PSA level were observed with vitamin D supplementation from baseline to follow up as compared to placebo. However, while 63% of the historical controls exhibited progression as defined by an increase in the number of positive biopsy cores or in Gleason score, only 34% of those supplemented with vitamin D<sub>3</sub> progressed<sup>98</sup>. These findings therefore indicate that some benefit may be attained from vitamin D supplementation in men with prostate cancer, but a larger randomized, double-blind clinical trial would be required to confirm these results.

Regarding mortality, studies conducted to date of 25(OH)D and prostate cancer mortality have been equivocal, with some studies showing a significant inverse association<sup>91,99,100</sup> and others being null<sup>88,95,101,102</sup>. Therefore, there is no convincing evidence that vitamin D will prevent prostate cancer, some evidence that it may prevent progression of early-stage disease, and inconsistent findings for mortality.

Overall, association studies of circulating concentrations of 25(OH)D and colorectal, breast, and prostate cancer incidence, progression, or mortality have yielded a wide range of results of varying consistency. As shown in Figure 1, the evidence is strongest for an association between 25(OH)D and colorectal adenoma incidence, colorectal cancer incidence and mortality, and breast cancer progression and/or mortality. However, data from randomized, controlled clinical trials completed to date generally provide no support for the findings of the association

studies. This highlights some major challenges for the field, including several integral challenges regarding the employment of 25(OH)D as a biomarker for vitamin D, as discussed below.

The first question raised by disparate findings of association studies as compared to clinical trials is whether circulating concentrations of 25(OH)D act as an appropriate biomarker for vitamin D. It is possible that in addition to serving as a biochemical marker for vitamin D intake and endogenous synthesis, 25(OH)D captures other important information about an individual's risk factor profile that either directly or indirectly is itself associated with health outcomes<sup>59</sup>. As mentioned previously, such characteristics that have been well-documented to be related to 25(OH)D levels include body size, physical activity, genetic background, and skin pigmentation<sup>59</sup>. For example, an individual with a lower BMI and higher physical activity levels is more likely to have higher 25(OH)D concentrations than a person with a high BMI who exercises less frequently. However, because both a smaller body size and greater physical activity are both related to lower cancer risk, it is difficult to separate the effects of these characteristics from those that may be attributed to 25(OH)D levels. As such, we previously hypothesized that 25(OH)D may act as a biomarker for a healthier lifestyle itself that may be related to a lower risk for cancer<sup>59</sup>. Although the majority of the studies reviewed herein have statistically controlled for at least some of these variables, the potential for residual confounding remains a major challenge for epidemiological studies<sup>103</sup>.

The second major question relates to the translation of laboratory experiments of vitamin D and cancer to human populations. Specifically, laboratory work has convincingly shown that 1,25(OH)<sub>2</sub>D elicits potent anti-carcinogenic effects including inhibition of cellular proliferation and growth and induction of differentiation in cancer cells<sup>104,105,106,107</sup>. However, as mentioned above, historically 1,25(OH)<sub>2</sub>D was rarely measured in large epidemiological studies due to both technical measurement challenges and because it is

subject to rigid homeostatic control and thus exhibits less variation in human populations<sup>108</sup>. Individual characteristics that are associated with circulating concentrations of 25(OH)D do not appear to have the same relationship with levels of 1,25(OH)<sub>2</sub>D<sup>109</sup>; however, recent work has demonstrated that supplementation with 4000 IU/d of vitamin D<sub>3</sub> significantly increases 1,25(OH)<sub>2</sub>D concentrations in the blood<sup>98</sup>, suggesting that these levels may be more responsive to intake than was perhaps previously thought. Nonetheless, local synthesis of 1,25(OH)<sub>2</sub>D at the cellular level has been established to be of key importance in any anti-carcinogenic effect of the hormone<sup>110</sup>. Colon carcinoma (Caco-2) cells possess 25-hydroxyvitamin D<sub>3</sub> 1-alpha hydroxylase (CYP27B1) activity<sup>111</sup>, and this enzyme is responsible for the hydroxylation of 25(OH)D to form 1,25(OH)<sub>2</sub>D. CYP27B1 expression is increased in early colon tumors, but is partially or fully inhibited as colon cells become undifferentiated<sup>112</sup>; while the 1,25(OH)<sub>2</sub>D catabolizing enzyme (CYP24A1) is increased in colon cancer cells<sup>111</sup>, suggesting that the amount of cellular 1,25(OH)<sub>2</sub>D available as cancer progresses is substantially suppressed. In addition, we have shown that genetic variants in these enzymes markedly affect the cellular activity of CYP27B1 and CYP24A1<sup>113</sup>. As such, even if 1,25(OH)<sub>2</sub>D may potentially provide information about systemic availability of the hormone, it is unlikely to capture data regarding the localized, cellular synthesis of 1,25(OH)<sub>2</sub>D.

Another potential challenge to studying the effects of vitamin D in epidemiological work is related to interactions with calcium, which itself has been linked to cancer outcomes<sup>114,115</sup>. The vitamin D metabolite 1,25(OH)<sub>2</sub>D has a critical role in calcium homeostasis<sup>116</sup>; a decrease in calcium results in secretion of parathyroid hormone, which in turn results in increased production of 1,25(OH)<sub>2</sub>D<sup>117</sup>. Therefore, greater intake of calcium in the diet may in fact suppress the production of 1,25(OH)<sub>2</sub>D at the cellular level, which would in turn attenuate any chemopreventive effects of this metabolite.

Pre-cancerous lesions		Incidence	Progression	Mortality
Incident	Recurrent	Colorectal Cancer	Colorectal Cancer	Colorectal Cancer
		Breast Cancer	Breast Cancer	
		Prostate Cancer	Prostate Cancer	

**Figure 1.** Summary of findings from observational epidemiological studies of 25(OH)D in the carcinogenesis pathway of colorectal, breast, and prostate cancers. Solid-colored bars represent consistent evidence for protection against cancer, white bars represent no evidence, and hashed bars represent inconsistent evidence for the association between 25(OH)D and the indicated endpoints of pre-cancerous lesions and cancer incidence, progression, or mortality.

In summary, after more than two decades of research into the association of vitamin D and cancer, results of association studies between 25(OH)D and colorectal, breast, and prostate cancer have indicated consistent inverse relationships for colorectal adenoma incidence, colorectal cancer incidence and mortality, and for breast cancer progression and/or mortality. In contrast, randomized, double-blind clinical trials conducted to date have failed to support these findings. Until the results of ongoing clinical trials are reported, it will be difficult to ascertain if vitamin D itself is related to a reduction in risk for some cancer endpoints, or whether high concentrations of the vitamin D biomarker 25(OH)D serve as a marker for an overall beneficial risk factor profile<sup>59,118</sup>.

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## Competing Interests

The authors have declared that no competing interest exists.

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