Vitamin D and clinical cancer outcomes: a review of meta-analyses

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Running title: Vitamin D and cancer: clinical data

Text word count (text only): 4342 81 references 2 Tables 0 Figures

Appendix: 6 Tables, 0 Figures

Abstract word count: 260

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jbm4.10420

Abstract

The relationship between vitamin D status or supplementation and cancer outcomes has been examined in several meta-analyses. To address remaining knowledge gaps, we conducted a systematic overview and critical appraisal of pertinent meta-analyses. For meta-analyses of trials, we assessed their quality using AMSTAR-2 (A Measurement Tool to Assess Systematic Reviews), strength of associations using umbrella review methodology and credibility of evidence using GRADE (Grading of Recommendations, Assessment, Development and Evaluation) criteria. Meta-analyses of observational studies reported inverse associations of 25-hydroxyvitamin D with risk of cancer incidence and cancer mortality and, particularly for colorectal cancer, fulfilled some of Bradford-Hill's causation criteria. In meta-analyses of trials, vitamin D supplementation did not affect cancer incidence. However, we found credible evidence that vitamin D supplementation reduced total cancer mortality risk, with 5 out of 6 meta-analyses reporting a relative risk (RR) reduction of up to 16%: RR=0.84 (95% confidence interval: 0.74-0.95). The strength of the association, however, was classified as weak. This was true among meta-analyses of high, moderate and lower quality (AMSTAR-2-rated). Trials did not include large numbers of vitamin D-deficient participants, many tested relatively low doses and lacked sufficiently powered data on site-specific cancers. In conclusion, meta-analyses show that, while observational evidence indicates that low vitamin D status is associated with a higher risk of cancer outcomes, randomized trials demonstrate that vitamin D supplementation reduces total cancer mortality but not cancer incidence. However, trials with larger proportions of vitamin D-insufficient

participants and longer durations of follow-up, plus adequately powered data on site-specific common cancers, would provide further insight into the evidence base.

Keywords: Vitamin D; circulating 25-hydroxyvitamin D; cancer incidence; cancer mortality; meta-analysis; randomized controlled trial

Introduction

Ecological studies of cancer incidence and mortality have shown that sun exposure, especially solar ultraviolet-B (and hence vitamin D production), is associated with reduced risk of many cancer types (1-3). This supports the hypothesis that vitamin D may have a beneficial impact on cancer outcomes, which is further strengthened by the identification of biological mechanisms that may explain these associations (4) and prospective observational studies which demonstrate that dietary intake of vitamin D (including vitamin D supplementation) is associated with a reduced risk of cancers, although confounding is important to consider (5-8).

Meta-analyses provide an opportunity to make decisions on accurate, succinct, credible and comprehensive summaries of the best available evidence, and act as a key tool for healthcare professionals to achieve evidence-based decisions (9). Several meta-analyses have synthesized data on the association of vitamin D status with cancer outcomes from observational studies (5-7, 10-14) and the effect of vitamin D supplementation on cancer outcomes in randomized clinical trials (15-22). However, the evidence base is inconsistent and fragmented into various meta-analyses that combine different study populations and outcomes, making assessment of the evidence using a similar methodological framework difficult. Further, as the methodologic quality of vitamin D-cancer meta-analyses may vary, uncritically accepting their results carries risk (9).

Thus, we decided to conduct a systematic overview, and critical appraisal of pertinent meta-analyses to better characterize the evidence on vitamin D status or supplementation in relation to cancer outcomes. In our critical appraisal, particular attention was given to

intervention studies as this study design, being the gold standard for effectiveness research, provides the highest relevance for evidence-based decision making. For this, we systematically assessed the quality of meta-analyses, plus strength and credibility of the evidence from these studies across multiple cancer outcomes, and discussed differences between meta-analyses. Finally, we discussed limitations of the evidence presented and provided some future research directions.

Methods

We searched Medline and PubMed for articles published up until 12 May 2020, using the following search terms: “vitamin D”, “cancer” and “meta-analysis”. No language restrictions were applied. This was supplemented by a manual search of reference lists from identified articles.

The quality of meta-analyses was assessed using AMSTAR-2 (A Measurement Tool to Assess Systematic Reviews), a 16-point assessment tool of the methodological quality of systematic reviews (9). Of the 16 domains, items 2, 4, 7, 9, 11, 13 and 15 are considered “critical” domains (can critically affect the validity of a review and its conclusion) (9). Based on weaknesses in the critical and non-critical domains, the overall confidence in the results of the meta-analysis was classified as “high”, “moderate”, “low”, or “critically low” (9). AMSTAR-2 has good inter-rater agreement, test-retest reliability and content validity (9).

The strength of associations was evaluated based on umbrella review criteria (23). For this, small-study effects were evaluated using Egger’s test (24) and the excess significance test was applied (excess significance was claimed at $P \leq 0.10$) (25, 26). Based on these criteria

findings, we classified the strength of the association (effect) as “convincing”, “highly suggestive”, “suggestive” or “weak” (Appendix Table 1) (23). Associations were considered non-significant if the P-value was >0.05. To provide a practical metric of the efficacy of vitamin D supplementation, the number needed to treat was calculated (27).

The credibility of pooled estimates of meta-analyses was qualitatively assessed using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) method (28). For each outcome, GRADE produces a credibility of estimate and summary of findings in a table that is easily understandable for study participants, policy makers, researchers, guideline developers and other interested stakeholders (scoring detailed in Appendix Table 2) (28). AMSTAR-2 and GRADE assessments were performed by one researcher (J.D.S.) and verified by another (R.S.), and discrepancies were discussed and resolved by consensus.

Results

Meta-analyses of observational studies

We found 35 meta-analyses that investigated relationships between vitamin D status, as measured by circulating 25-hydroxyvitamin D (25(OH)D), and cancer outcomes: 29 on cancer incidence (5, 6, 11, 14, 29-53), 3 on cancer mortality (10, 54, 55) and 3 on both (13, 56, 57) (Table 1).

Cancer incidence

With total cancer incidence as the outcome, one of these meta-analyses combined data from 8 prospective cohort studies (70,018 participants and 7511 events) (13). The summary relative risk (RR) estimate of the highest 25(OH)D category compared to the lowest was 0.86 (95% confidence interval (CI): 0.73-1.02), indicating a marginal relationship in the inverse direction, with a significant between-study heterogeneity (I^2=70.8%). Dose-response analysis indicated a 7% reduction in risk (RR=0.93; 0.91-0.96) per 20 nmol/L increment in 25(OH)D.

There were numerous meta-analyses (43 from 32 articles) on site-specific cancer outcomes: breast (12 meta-analyses), colon (n=3), rectum (n=3), colorectal (n=9), colorectal adenomas (n=4), prostate (n=4), kidney (n=1), liver (n=1), lung (n=1), non-Hodgkin's lymphoma (n=1), ovarian (n=1), pancreatic (n=1) and thyroid (n=2). Most of these were prospective: cohort studies or case-control studies nested within these. The vast majority of associations were in the inverse direction. Of these, significant relationships were observed in most meta-analyses of breast cancer (9 out of 12) and all meta-analyses of colorectal cancer. Generally, heterogeneity was high for breast cancer (I^2 up to 91%) but low to moderate for colorectal cancer (e.g., I^2 was 0-9% in 3 meta-analyses). There were strong associations for some cancers: breast (lowest RR=0.41 (33)), colorectal (lowest RR=0.49 (38)) and rectum (lowest significant RR=0.50 (37)). Sizeable relationships were observed too for colorectal adenoma (lowest OR=0.59 (40)). Dose-response meta-analyses reported inverse trends for breast (34, 49, 56), colon (6), colorectal (39, 48), liver (51) and lung (11) cancers.

Cancer mortality

A recent meta-analysis had total cancer mortality as the outcome (13). This report combined data from 16 prospective cohort studies on 101,794 participants without cancer at baseline, 8729 of whom had a cancer-related death. The pooled RR – of a highest-versus-lowest 25(OH)D group comparison – was 0.81 (95% CI: 0.71-0.93), indicating an inverse association, with moderate heterogeneity (I^2=48.8%). Dose-response analysis (of studies with ≥3 categories of 25(OH)D) revealed that the risk of cancer mortality was reduced by 2% (RR=0.98; 95% CI: 0.97-0.99) with each 20 nmol/L increment of 25(OH)D.

Four meta-analysis articles of prospective studies focused on cancer patients (10, 54-56). They reported sizeable inverse associations between 25(OH)D quantile (highest versus lowest) and cancer-specific mortality: pooled hazard ratios were 0.50-0.65 (in breast cancer, colorectal cancer and lymphoma patients) in one study (54), summary RR=0.58 for breast cancer mortality in another study (56), pooled RR ranged from 0.57 (for breast cancer mortality) to 0.65 (for colorectal cancer mortality) in another (10) and the summary hazard ratio was 0.73 (for colorectal cancer mortality) in the fourth study (55). One of these studies reported an inverse dose-response association (56).

Meta-analyses of randomized clinical trials

We identified 8 meta-analyses of clinical trials that evaluated the impact of vitamin D supplementation on cancer outcomes (incidence and mortality; Table 2). Appendix Table 3 summarizes their study selection criteria, which excluded studies based on several factors such as co-administration of calcium that differed across the treatment groups (16, 19), treatment with hydroxylated vitamin D or vitamin D analogs (15, 17, 20, 21), duration of

follow-up (<1 year (19, 20)) or intervention (<3 years (22), age (<18 (21) or <60 (19) years), number of outcomes (<10 (20)) and pregnancy (15, 16, 21).

Quality of meta-analyses

The AMSTAR-2 ratings of the 8 meta-analyses are summarized in Table 2 and detailed in Appendix Table 4. Five meta-analyses had shortcomings in critical domains (17-20, 22): all 5 did not cite a pre-defined, registered protocol, 4 did not cite excluded primary studies (18-20, 22), 3 did not use a satisfactory technique to assess risk of bias (17, 18, 20) and 2 used a literature search that was not fully comprehensive (17, 18). All studies had deficiencies in non-critical domains, with the most common being reporting funding sources of primary studies (absent in 7 meta-analyses (15, 17-22)) and explaining selection of study designs for inclusion in the review (absent in 6 meta-analyses (15-17, 19, 21, 22)). Based on weaknesses in the critical and non-critical domains, the overall confidence in the 8 meta-analyses was deemed critically low or moderate for 7 of these (15, 17-22).

Cancer incidence

Six of these meta-analyses had total cancer incidence as an outcome (16-20, 22). These reports were published between 2014 and 2019 – with each comprising 4-18 studies, 18,440-83,353 participants and 1061-6537 cancer incident events. The pooled RR effects in these reports were similar, ranging from 0.98 (10 studies) (20) to 1.03 (24 studies) (19), with all 95% confidence intervals encompassing 1. The I^2 for these effects was 0% (16-20) or 31% (22), indicating no significant heterogeneity.

Two of these meta-analyses had specific cancer incidence as an outcome (15, 16). In these, the pooled RR effects were 0.97 (7 studies) to 1.11 (2 studies) for breast cancer, 0.86 (95% CI: 0.69-1.07) for lung cancer (5 studies), 1.11 (95% CI: 0.92-1.34) for colorectal cancer and 0.91 (95% CI: 0.57-1.46) for pancreatic cancer (2 studies).

Cancer mortality

Six of these meta-analyses had cancer mortality as an outcome (16, 18-22). The pooled RR effects in these reports were similar and all in the inverse direction, ranging from 0.84 (95% CI: 0.74-0.95; 12 studies) to 0.88 (95% CI: 0.70-1.09; 24 studies). The 95% CIs did not encompass 1 in 5 out of 6 of these meta-analyses (16, 18, 20-22), indicating consistently beneficial impacts on cancer mortality.

Strength and credibility of meta-analysis effects

Five intervention meta-analyses of total cancer mortality had weak strength of association according to umbrella review criteria (16, 18, 20-22), with all scoring high with GRADE and with NNT values ranging from 86 to 381. This was consistent among meta-analyses of high, moderate and lower quality (AMSTAR-2-rated). The remaining meta-analyses reported non-significant effects, with GRADE credibility that was moderate (16, 17) to high (18-20, 22) for total cancer incidence and low (15, 16) to moderate (16) for site-specific cancer incidence. A breakdown of these scores is provided in Appendix Tables 5 and 6.

Differences between trial meta-analyses

As pooled effects across meta-analyses on the same outcome were mostly similar (with overlapping 95% confidence intervals; Table 2) despite variable study selection criteria (Appendix Table 3), this enhances the external validity of our findings that vitamin D reduces total cancer mortality (in nearly all meta-analyses) but not cancer incidence. Where pooled effects for the same outcome variations did vary, this can be explained by differences in the number of primary studies in meta-analyses, which is influenced by publication year (Table 2) and study selection criteria. The most important variation occurred for cancer mortality, in which the Goulão *et al* (19) meta-analysis did not find a significant overall effect but the remaining 5 meta-analyses did (Table 2). Goulão and colleagues (19) excluded primary studies in which calcium was co-administered with vitamin D but not with placebo, which allowed it to disentangle calcium and vitamin D effects. This restriction meant that the Women's Health Initiative (58), in which calcium was given with vitamin D in the intervention group only, was not included in this meta-analysis (unlike nearly all other ones (16, 18, 20, 22)). However, this exclusion may not have been important for validity if calcium did not influence cancer mortality, as suggested by prior studies (59). Also, the number of participants (n=11202) and events (n=320) in the Goulão *et al* (19) meta-analysis was substantially lower than those in the other meta-analyses (n=44290-75239 and n=939-1192 for number of participants and events, respectively; Table 2). This is because two large (n>5000) trials in addition to the Women's Health Initiative (58) – the ViDA (60) and VITAL (61) studies – were included in other meta-analyses (showing vitamin D benefits) (20-22) but not in the Goulão *et al* (19) one as they were published after it (19). Thus, the Goulão *et al*

(19) meta-analysis did not capture effects from some large trials and had reduced statistical power.

Limitations of meta-analyses and their primary studies

Owing to several limitations, the meta-analyses and their primary studies have boundaries of applicability. These limitations influence the strength of associations and are key targets for future research. We discuss these for observational and intervention studies separately.

Observational studies

A limitation of the observational studies we reviewed is that, although they adjusted for multiple confounders, they are vulnerable to residual confounding; more so when the confounders (e.g., smoking behaviour, body mass index, physical activity, diet) are measured less well. Adding to this issue is that vitamin D status is related to multiple diseases, besides cancer (62). Thus, 25(OH)D-cancer associations do not fulfil one of Bradford-Hill's causation criteria, specificity (63), raising the possibility that confounding may be important. On the other hand, confounding may not entirely explain these associations as risk factors can cause more than one disease (63) and vitamin D has pleiotropic cellular effects (62). A second limitation is that several observational studies, especially those on breast cancer incidence (14, 29, 30, 32, 33, 35), were case-control investigations – and are thus susceptible to reverse causation since 25(OH)D measurement was performed in those already diagnosed with cancer and low vitamin D status may be a consequence of the disease rather than a cause. For instance, during cancer therapy or when symptoms are severe, sunlight exposure, physical

activity and dietary habits are likely to change (because of hospitalizations, disability or lifestyle changes), and cancer-associated inflammation may depress 25(OH)D (64). However, this problem was avoided in prospective studies where blood samples were collected well before cancer diagnosis. The studies in the meta-analyses we reviewed were mainly prospective (nested case-control or cohort) and we observed inverse associations in them, suggesting that 25(OH)D may affect cancer (Table 1; rather than vice versa). Third, several observational studies are prone to 25(OH)D measurement error as they did not measure vitamin D status with the gold standard laboratory method for 25(OH)D measurement, liquid chromatography-tandem mass spectrometry (65). Fourth, whereas many observational studies were of high quality, as assessed by the Newcastle-Ottawa Scale, several were of suboptimal (medium) quality, which may have attenuated 25(OH)D-cancer associations. In support of this, a recent meta-analysis reported that 25(OH)D was inversely associated with total cancer incidence and mortality in studies of high quality but not in those of medium quality (13). Fifth, while most meta-analyses had low or moderate between-study heterogeneity (I^2<50% or P-values>0.05) (6, 31, 36, 38, 39, 42, 44, 45, 47, 49, 50, 52-54, 56, 57), some did not and thus their results should be interpreted with caution. These include meta-analyses on colorectal adenoma and cancer occurrences of the breast, rectum and all types combined (5, 6, 13, 14, 29, 30, 32, 35, 36) – which reported high between-study heterogeneity ($I^2\geq$50%). Finally, most participants in the observational studies were of white ethnicity, which limits generalizability of findings to non-white ethnic groups.

Randomized clinical trials

An important issue for intervention studies is the growing RCT evidence that health benefits of vitamin D supplementation are greatest in vitamin D-deficient people (66). This may also apply to cancer as a recent meta-analysis of cohort studies revealed that 25(OH)D was inversely associated with cancer incidence at <~30 nmol/L only and had an inverse relationship with cancer mortality that was strongest at low 25(OH)D levels (especially <~50 nmol/L) (13). Thus, any cancer-related benefits of vitamin D supplementation may be greatest in vitamin D-deficient individuals. However, trials have not contained large numbers of such people. For example, the average baseline 25(OH)D of 10 trials examined in the recent meta-analysis by Keum *et al* (20) we reviewed was ~60 nmol/L.

Insufficient vitamin D dose may be another limitation. For example, in the Goulão *et al* meta-analysis of cancer incidence (19), the daily dose equivalent (dose divided by days between each dose) in multiple primary studies (n=7) was relatively low (<1000 IU/day), raising a question of whether a higher dose may have produced a different (stronger) effect. A related issue is the frequency of the dosing regimens. Most trials have utilised daily dosing, while evidence is limited for supplementation administered monthly or weekly (20).

Third, as carcinogenesis is a long-term and gradual process (often spanning decades), the need for a long follow-up period is particularly great (67). The importance of this is reflected in the long follow-up periods of 25(OH)D-cancer cohort studies, such as those in the Han *et al* meta-analysis (of total cancer incidence and mortality) (13), which were 12-13 years on average and up to 28 years. However, most of the primary studies included in our meta-analyses of trials had follow-up periods of no more than 5 years and this may have been insufficient to detect effects on cancer (67). In support of this, the meta-analysis by Zhang *et*

al (21) we reviewed found that benefit of supplementation on reduced cancer mortality was observed in trials with longer follow-up (>3 years) but not in those with a shorter follow-up.

A fourth limitation is that, as shown in the meta-analyses for observational studies, 25(OH)D was more consistently associated with colorectal and breast cancers than other cancers (Table 1). Thus, there may be stronger effects of supplementation against certain cancer types or, possibly, residual confounding may be greater for some cancers than others (e.g., confounding by BMI, physical activity, and diet may be of particular relevance to studies of vitamin D and colorectal cancer). However, whereas the cancer outcomes that have dominated our RCT findings are those based on all cancers combined (Table 2), results for site-specific cancers are lacking. Almost all trials that evaluated impacts on site-specific cancers did not include these as primary endpoints and data on rarer cancers (e.g., kidney) are missing.

Fifth, the vast majority of participants in the intervention studies were white, which restricts applicability of findings to non-white populations.

Finally, there were quality-related shortcomings of the meta-analyses of intervention studies we reviewed. The most common ones, detected by AMSTAR-2, were the lack of information on funding sources of primary studies (noteworthy as many vitamin D trials are industry-funded and have a high risk of "for-profit" bias (16)), of an explanation of study design selection and of a review protocol describing pre-specified methodology. Most of these meta-analyses were published prior to the availability of AMSTAR-2 reporting standards (in 2017 (9)), which may have contributed to their lower AMSTAR-2 ratings.

However, as mentioned above, our vitamin D-cancer findings for meta-analyses of high and moderate quality (AMSTAR-2-rated) were similar to those of lower quality.

Limitations of current review

A potential limitation is that, although a comprehensive and systematic literature search was performed, we may have missed some meta-analyses. Second, our study was a meta-review and, while this provides an overarching perspective on a research topic, we did not provide granulate analyses at the primary study level. Third, our review focuses on meta-analyses and thus some primary studies may not have been included either because the meta-analysis did not identify them or they were too recent to be included. Finally, we did not critically appraise the quality of all primary studies individually as this should be done in each meta-analysis and doing this was beyond the scope of our review.

Future research

Several areas of future research would strengthen our understanding of vitamin D effects on cancer. First, given the emerging evidence for threshold effects related to vitamin D status, future trials should aim to recruit participants with vitamin D insufficiency (25(OH)D<50 nmol/L). There are major logistical and practical barriers to doing this in populations that are vitamin D replete, and trials could be undertaken more easily and cheaply in populations with a high prevalence of vitamin D insufficiency. These trials should have longer follow-up periods, include more adequately powered data on site-specific cancers (feasible for common cancers) and study more non-white populations. However, ethical issues can arise with the

conduct of long-term trials in vitamin D-deficient participants, as 50% will be randomly assigned to placebo and remain deficient for a prolonged period.

Second, cells that express the cell surface receptor proteins megalin and cubulin (e.g., those in the kidney, lung, thyroid, mammary gland, gall bladder and thyroid) can internalise 25(OH)D bound to vitamin D-binding protein, with subsequent unbinding of 25(OH)D intracellularly and conversion to 1,25-dihydroxyvitamin D, which can exert anticancer effects by activating the vitamin D receptor (68, 69). In contrast, 25(OH)D entry in cells not expressing the megalin-cubulin receptor is proposed to occur via diffusion of unbound, free 25(OH)D across the cell membrane (68, 69). However, the studies we reviewed measured total 25(OH)D, comprising not only free (and bioavailable) 25(OH)D, but mostly (~90%) 25(OH)D that is bound tightly to vitamin D-binding protein and thus may not get into some cancer cells easily (70). As evidence of importance, a large, prospective cohort study reported that higher bioavailable, rather than total, 25(OH)D levels were independently associated with improved survival in patients with hepatocellular carcinoma (71). Further, in a recent RCT of patients with digestive tract cancer, vitamin D supplementation improved 5-year relapse-free survival in those with low bioavailable 25(OH)D, but not in those with high bioavailable 25(OH)D (70) or in those with low total 25(OH)D (<20 ng/mL) (72). These studies suggest that, for some cancer types, free and bioavailable 25(OH)D may better assess true vitamin D status (and deficiency) than total 25(OH)D in future trials.

Third, articles that reported inverse, longitudinal associations between 25(OH)D and cancer mortality (10, 54-56) (Table 1) were of cancer patients and thus suggest a potential role of vitamin D in cancer therapy – as a opposed to cancer prevention, the focus of the other

articles we reviewed. RCT data investigating vitamin D as a cancer treatment are scarce, with one relatively small RCT (n=139) reporting an improvement in median progression-free survival or death (hazard ratio=0.64) over 22.9 months (median) in colorectal cancer patients (73) and another (n=417) reporting an age-adjusted benefit on relapse-free survival (hazard ratio=0.66) over 3.5 years (median) in patients with digestive tract cancers (72). However, further trials are required in this research area, ideally with longer follow-up (74). Including biological measurements would help understand underlying mechanisms and this requires considering not only antineoplastic influences, but broad biological effects too, as 25(OH)D is inversely related to all-cause mortality in patients with or without cancer (74).

Fourth, cancer incidence and mortality, and overall survival, though considered the gold standard endpoint in oncology trials, require a large sample size and long follow-up time to achieve adequate statistical power (75). In comparison, other clinical endpoints can be assessed earlier, and thus could be measured in parallel. One of these is health-related quality of life, which is considered an outcome in assessing clinical benefit and has emerged as a primary endpoint in oncology clinical trials (75). Encouragingly, observational cohort studies found that, in cancer patients, vitamin D intake and 25(OH)D predict improved quality of life (76-78), but RCT data are needed to evaluate effects on this outcome. Another is tumour-centred endpoints, such as progression-free survival, disease-free survival, tumour response and circulating tumour cells (75). As mentioned, two RCTs reported beneficial effects on median progression-free survival (73) and relapse-free survival (72), but further RCTs are needed (74). Thus, adding these other clinical endpoints (alongside gold standard outcomes)

to future vitamin D trials would provide an earlier assessment of and more comprehensive evaluation of efficacy (75).

Finally, a novel area of research is investigating whether vitamin D pathway genes may alter health effects on vitamin D supplementation. A meta-analysis of 8 prospective studies reported that colorectal cancer risk was lower in participants with the BB genotype of the *BsmI* vitamin D receptor single-nucleotide polymorphism (6). A recent RCT found that vitamin D receptor genotypes modified the effect of vitamin D supplementation on the prevention of advanced colorectal adenomas (79). Specifically, vitamin D supplementation reduced risk by 64% among those with the *rs7968585* genotype and increased risk by 41% among those with 1 or 2 G alleles (79). Such work helps identify who may benefit from supplementation for cancer prevention based on vitamin D-related genotypes. Given the knowledge gap in investigating vitamin D pathway genotypes as modifiers of effects on cancer outcomes, assessment of these in recent trials (possibly as IPD meta-analyses) and further trials in this research area are both warranted. As recognition of the importance of this, the VITAL trial (61), for example, is in the process of conducting such analyses of gene variants (JE Manson, personal communication, 2020).

Conclusion

Observational studies showed that, in many cases, low vitamin D was inversely associated with cancer outcomes. For this, the associations for some outcomes, particularly colorectal cancer, seem to fulfil some (but not all) of Bradford-Hill's criteria for causation (63), including consistency of findings across different meta-analyses and primary studies,

temporality (prospective-study associations), biological gradient (dose-response associations) and strength of associations (strong in some cases).

To our knowledge, this review is the first report to systematically compile and appraise clinical evidence – by concurrently using AMSTAR-2, umbrella review and GRADE assessment tools – of vitamin D supplementation in relation to cancer outcomes from meta-analyses. We found highly credible RCT evidence that vitamin D supplementation reduces risk of total cancer mortality, but the magnitude of effect was classified as weak. Our finding of a highly credible weak effect on total cancer mortality is line with that of a 2017 systematic review of meta-analyses which reported that they indicate that vitamin D supplementation reduces risk of all-cancer mortality (80). We extend that review by including more recent meta-analyses in our assessment (13, 19-21) and by critically evaluating the evidence for this outcome using the abovementioned appraisal tools.

The available research, however, is not without limitations. To address these limitations and to provide clearer and further insight into the role of vitamin D in cancer incidence and related mortality, future research should include having trials with more vitamin D-insufficient participants and of longer follow-up duration, plus adequately powered data on site-specific cancers (where feasible).

Acknowledgements: The Health Research Council of New Zealand (HRC) supported J.D.S. with a fellowship. HRC had no role in the design, analysis, interpretation or presentation of the results.

Authors' roles: J.D.S conducted the literature search, created the tables and drafted the manuscript. R.S. verified the AMSTAR-2 and GRADE assessments. J.E.M. and R.S. edited and revised the manuscript. All authors approved the final version of the manuscript.

Conflict of interest: None declared.

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Table 1. Meta-analyses of cohort studies on the association between 25-hydroxyvitamin D and cancer outcomes

First author, publication year	Studies & design (n)	Participants (n)	Events (n)	Unit of 25-hydroxyvitamin D comparison	Pooled association (95% CI)	I^2 (%) or P-value for heterogeneity*
Cancer incidence						
All						
Han, 2019 (13)	8 PC	70018	7511	Highest vs. lowest group	RR=0.86 (0.73-1.02)	71
				Per 20 nmol/L (dose-response)	RR=0.93 (0.91-0.96)	NR
Breast						
Chen, 2010 (29)	7 (4 CC, 3 NCC)	11330	5489	Highest vs. lowest quartile	OR=0.55 (0.38-0.80)	86
Yin, 2010 (30)	9 (5 CC, 4 NCC)	12901	6147	Per 20 ng/mL	OR=0.73 (0.60-0.88)	84
	4 NCC	6327	3117		OR=0.92 (0.83-1.04)	NR
Chung, 2011 (31)	4 NCC	4726	2363	Per 10 nmol/L (dose-response)	OR=0.99 (0.97-1.01)	NR
Gandini, 2011 (32)	10 (1 PC, 4 NCC, 5 CC)	29742	6175	Per 10 ng/mL	RR=0.89 (0.81-0.98)	88
	5 (1 PC, 4, NCC)	23078	3145		RR=0.97 (0.92-1.03)	54
Mohr, 2011 (33)	11 (6 NCC, 5 CC)	16337	7547	Highest vs. lowest quintile	Peto OR=0.61 (0.47-0.80)	P<0.0001
	6 NCC	9673	4517	Highest vs. lowest quintile	Peto OR=0.87 (0.77-0.99)	P=0.50
	5 CC	6664	3030	Highest vs. lowest quintile	Peto OR=0.41 (0.31-0.56)	P=0.005
Bauer, 2013 (34)	Pre-menopause: 6 PC	1613	2890	Per 5 ng/mL (dose-response)	RR=0.99 (0.97-1.04)	NR
	Post-menopause: 9 PC	3929	8766		RR=0.97 (0.93-1.00)	NR
Chen, 2013 (35)	21 (10 NCC, 1 RSP, 10 CC)	26317	11771	Highest vs. lowest quartile	OR=0.52 (0.40-0.68)	89
	11 NCC/RSP	6811	15852	Highest vs. lowest quartile	OR=0.86 (0.75-1.00)	40
Wang, 2013 (49)	14 (1 PC, 13 NCC)	25354	9110	Highest vs. lowest group	RR=0.84 (0.75-0.95)	38
	11 (1 PC, 10 NCC)	20252	6715	Per 10 ng/mL (dose response)	RR=0.97 (0.94-0.99)	P=0.13
Kim, 2014 (56)	14 (1 PC, 13 NCC)	27534	9526	Highest vs. lowest group	RR=0.92 (0.83-1.02)	27
				Per 10 ng/mL (dose response)	RR=0.98 (0.96-1.00)	P-value: NS
Estébanez, 2018 (50)	29 (14 NCC, 15 CC)	58855	18358	High vs. low group	OR=0.66 (0.57-0.76)	41
	14 NCC	24271	10266	High vs. low group	OR=0.92 (0.83-1.01)	16
	4 PC	16875	3350	Variable group comparisons	OR=0.85 (0.74-0.98)	4
Hossain, 2019 (5)	14 (12 NCC, 1 CC, 1 MR)	123044	25515	Per 10 ng/mL	OR=0.99 (0.98-1.00)	79
	5 CC	2796	1306	<10 ng/mL vs. ≥10 ng/mL	OR=1.91 (1.51-2.41)	83
Song, 2019 (14)	40 (4 PC, 36 CC)	162322	31157	Per 5 nmol/L	OR=0.94 (0.93-0.96)	91

Colon						
Yin, 2009 (36)	6 NCC	2081	759	Per 20 ng/mL	OR=0.78 (0.54-1.13)	45
Lee, 2011 (37)	8 PC	4578	1822	Highest vs. lowest group	OR=0.77 (0.56-1.07)	P=0.04
Touvier, 2011 (6)	6 NCC	3550	1477	Per 100 IU/L (dose-response)	RR=0.95 (0.92-0.995)	48
Rectum						
Yin, 2009 (36)	4 NCC	719	258	Per 20 ng/mL	OR=0.41 (0.11-1.49)	63
Lee, 2011 (37)	9 NCC	4578	868	Highest vs. lowest group	OR=0.50 (0.28-0.88)	P=0.04
Touvier, 2011 (6)	5 NCC	1645	721	Per 100 IU/L	RR=0.95 (0.89-1.05)	67
Colorectal						
Gorham, 2007 (38)	5 NCC	1448	535	Highest vs. lowest group	Peto OR=0.49 (0.35-0.68)	P=0.90
Yin, 2009 (36)	5 NCC	3286	1199	Per 20 ng/mL	OR=0.57 (0.43-0.76)	9
Chung, 2011 (31)	9 NCC	2249	1127	Per 10 nmol/L	OR=0.94 (0.91-0.97)	NR
Gandini, 2011 (32)	9 (1 PC, 7 NCC, 1CC)	22948	2630	Per 10 ng/mL (dose-response)	RR=0.85 (0.79-0.91)	55
	8 (1 PC, 7 NCC)	22870	2604	Per 10 ng/mL (dose-response)	RR=0.85 (0.79-0.92)	59
Lee, 2011 (37)	8 NCC	4578	2690	Highest vs. lowest group	OR=0.66 (0.54-0.81)	P=0.04
Ma, 2011 (39)	9 (7 PC, 2 NCC)	6715	2767	Highest vs. lowest group	RR=0.67 (0.54-0.80)	0
				Per 10 ng/mL (dose-response)	RR=0.74 (0.63-0.89)	NR
Touvier, 2011 (6)	6 NCC	5833	2370	Per 100 IU/L	RR=0.96 (0.94-0.97)	0
Huang, 2019 (57)	30 (6 PC, 23 NCC, 1 CC)	204544	13051	Highest vs. lowest group	RR=0.68 (0.60-0.78)	56
Zhang, 2019 (48)	8 (1 NCC, 7 CC)	9594	2916	Highest vs. lowest group	OR=0.75 (0.58-0.97)	54
				Per 16 ng/mL (dose-response)	OR=0.79 (0.64-0.97)	54
Colorectal adenoma						
Wei, 2008 (81)	All adenomas:					
	7 (1 CS, 3 CC, 3 NCC/PC)	3787	2628	Highest vs. lowest quintile	OR=0.70 (0.56-0.87)	54
	Advanced adenomas:					
	2 NCC	1023	2347	High vs. low groups	OR=0.64 (0.45-0.90)	NR
Fedirko, 2010 (40)	3 CC	1386	616	Highest vs. lowest quartile	OR=0.59 (0.41-0.84)	NR
Yin, 2011 (41)	Incident events:	7654	3539	Per 20 ng/mL	OR=0.82 (0.69-0.97)	66
	9 (5 CC, 1 CS, 3 NCC)					
	Recurrent events: 3 PC	2169	984	Per 20 ng/mL	OR=0.87 (0.56-1.35)	57
Huang, 2019 (57)	22 (5 PC, 2 NCC, 14 CC, 1CS)	13652	6445	Highest vs. lowest group	RR=0.80 (0.71-0.89)	34
Prostate						

Yin, 2009 (46)	10 (1 PC, 9 NCC)	7806	3124	Per 10 ng/mL	OR=1.03 (0.96-1.11)	23
Chung, 2011 (31)	8 NCC	5609	2399	Per 10 nmol/L (dose-response)	OR=1.01 (0.99-1.04)	NR
Gandini, 2011 (32)	11 PC	26575	3956	Per 10 ng/mL(dose-response)	RR=0.99 (0.95-1.03)	37
Gilbert, 2011 (47)	14 (5 PC, 9 NCC)	12051	4353	Per 10 ng/mL	OR=1.04 (0.99-1.10)	0
Kidney						
Gallicchio, 2010 (42)	8 PC	1550	775	50-<75 vs. ≥100 nmol/L	OR=0.92 (0.44-1.92)	P-value: NS
Liver						
Guo, 2020 (51)	6 (1 PC, 5 NCC)	60811	992	High vs. low group	RR=0.78 (0.63-0.95)	54
				Per 10 nmol/L (dose-response)	RR=0.92 (0.89-0.95)	NR
Lung						
Feng, 2017 (11)	9 (6 PC, 3 CC)	111148	1511	Variable group comparisons	RR=0.84 (0.74-0.95)	50
				Per 10 nmol/L (dose-response)	RR=0.92 (0.87-0.96)	NR
Non-Hodgkin lymphoma						
Purdue, 2010 (43)	Males: 6 PC	923	733	>100 vs. 50-75 nmol/L	OR=0.67 (0.37-1.20)	NR
	Females: 4 PC	923	733	>100 vs. 50-75 nmol/L	OR=0.81 (0.39-1.69)	NR
Ovarian						
Yin, 2011 (44)	10 NCC	3373	884	Per 20 ng/mL	0.83 (0.63-1.08)	0
Pancreatic						
Stolzenberg-Solomon, 2010 (45)	6 NCC	833	345	≥100 vs. 50-75 nmol/L	OR=2.14 (0.93-4.92)	P>0.30
Thyroid						
Hu, 2018 (52)	9 (7 CC, 1 CS, 1 RSP)	7099	1172	<20 vs. ≥20 ng/mL	OR=1.42 (1.17-1.73)	27
	7 (5 CC, 2 CS)	6498	775	Cases vs. controls	SMD=-0.20 (-0.36, -0.03)	55
Zhao, 2019 (53)	6 CC	6241	711	Deficient vs. non-deficient	OR=1.30 (1.00-1.69)	38
	12 CC	7278	1239	Cases vs. controls	SMD=--0.37 (-0.45--0.28)	93
Cancer mortality						
All						
Li, 2014 (54)	Breast cancer patients: 4 PC	4813	661	Highest vs. lowest quartile	HR=0.65 (0.44-0.98)	45
	CRC patients: 3 (2 PC, 1 NC)	1558	883	Highest vs. lowest quartile	HR=0.65 (0.47-0.88)	6
	Lymphoma patients: 7 PC	1234	511	Highest vs. lowest quartile	HR=0.50 (0.36-0.68)	0
Han, 2019 (13)	16 PC	101794	8729	Highest vs. lowest group	RR=0.81 (0.71-0.93)	49

				Per 20 nmol/L (dose-response)	RR=0.98 (0.97-0.99)	NR
Breast						
Kim, 2014 (56)	4 PC	400	4556	Highest vs. lowest group	RR=0.58 (0.40-0.85)	27
	3 PC	NR	NR	Per 10 ng/mL (dose response)	RR=0.88 (0.79-0.98)	23
Maalmi, 2014 (10)	3 PC	2636	194	High vs. low group	HR=0.57 (0.38-0.84)	17
Colorectal						
Maalmi, 2014 (10)	3 PC	1558	566	High vs. low group	HR=0.65 (0.49-0.86)	0
Xu, 2018 (55)	5 (3 PC, 2 NCC)	4126	982	High vs. low group	HR=0.73 (0.55-0.97)	69
Huang, 2019 (57)	12 PC	53910	2021	High vs. low group	HR=0.64 (0.56-0.73)	3

*DerSimonian-Laird Q statistic. CC=case control; CI=confidence interval; CRC=colorectal cancer; CS=cross-sectional; HR=hazard ratio; MR=Mendelian randomisation; NCC=nested case-control; NR=not reported; NS=not-significant; OR=odds ratio; PC=prospective cohort; RR=relative risk; RSP=retrospective; SMD=standardized mean difference.

Table 2. Meta-analyses of intervention studies* on the effect of vitamin D supplementation on cancer outcomes

Study	Studies (n)	Sample size (n)	Events (n)	Pooled RR effect (95% CI)	NNT (95% CI)	I^2 (%)	Quality of meta-analysis (AMSTAR-2 rating)	Strength of association (umbrella review class)	GRADE credibility of evidence
Cancer incidence									
All									
Bolland, 2014 (17)	7	48167	3979	0.99 (0.93-1.05)	-	0	Critically low	NS	Moderate
Bjelakovic, 2014 (16)	14	49891	3851	1.00 (0.94-1.06)	-	0	High	NS	Moderate
Keum, 2014 (18)	4	45151	4333	1.00 (0.94-1.06)	-	0	Critically low	NS	High
Goulão, 2018 (19)	24	18440	1061	1.03 (0.91-1.15)	-	0	Critically low	NS	High
Haykal, 2019 (22)	9	42773	3022	0.96 (0.86-1.07)	-	31	Critically low	NS	High
Keum, 2019 (20)	10	83353	6537	0.98 (0.93-1.03)	-	0	Critically low	NS	High
Breast									
Sperati, 2013 (15)	2	5372	91	1.11 (0.74-1.68)	-	0	Moderate	NS	Low
Bjelakovic, 2014 (16)	7	43669	1135	0.97 (0.86-1.09)	-	0	High	NS	Moderate
Colorectal									
Bjelakovic, 2014 (16)	5	45598	436	1.11 (0.92-1.34)	-	0	High	NS	Moderate
Lung									
Bjelakovic, 2014 (16)	5	45509	329	0.86 (0.69-1.07)	-	0	High	NS	Moderate
Pancreatic									
Bjelakovic, 2014 (16)	2	36405	69	0.91 (0.57-1.46)	-	0	High	NS	Moderate
Cancer mortality									
All									
Bjelakovic, 2014 (16)	4	44492	1192	0.88 (0.78-0.98)	292 (159-1751)	0	High	Weak	High
Keum, 2014 (18)	3	44290	1190	0.88 (0.78-0.98)	86 (47-515)	0	Critically low	Weak	High
Goulão, 2018 (19)	7	11202	320	0.88 (0.70-1.09)	-	0	Critically low	NS	Moderate

Haykal, 2019 (22)	5	70547	1533	0.87 (0.79-0.96)	381 (236-1238)	0	Low	Weak	High
Keum, 2019 (20)	5	75239	1591	0.87 (0.79-0.96)	294 (182-957)	0	Critically low	Weak	High
Zhang, 2019 (21)	12	45578	939	0.84 (0.74-0.95)	279 (171-892)	0	Moderate	Weak	High

All were randomized controlled trials. NNT=number needed to treat; NS=non-significant association; RR=relative risk.

Appendix

Appendix Table 1. Umbrella review assessment grades*

Strength of association	Criteria
Convincing (Class I)	>1000 cases†
	Significant summary associations ($P<10^{-6}$) per random-effects calculations
	No evidence of small-study effects
	No evidence of excess of significance bias
	Prediction intervals not including the null value
	Largest study nominally significant ($P<0.05$)
	Not large heterogeneity ($I^2<50\%$)
Highly suggestive (Class II)	>1000 cases†
	Significant summary associations ($P<10^{-6}$) per random-effects calculations
	Largest study nominally significant ($P<0.05$)
Suggestive (Class III)	>1000 cases†
	Significant summary associations ($P<10^{-3}$) per random-effects calculations
Weak (Class IV)	Significant summary associations ($P<0.05$) per random-effects calculations
Non-significant association	Non-significant summary associations ($P>0.05$)

*From: Papatheodorou S. Umbrella reviews: what they are and why we need them. European Journal of Epidemiology. 2019;34:543–6.
†Total for the meta-analysis.

Appendix Table 2. GRADE assessment scoring*

Study design	Quality of evidence	Lower if	Higher if
Randomized trial ⟶	High	Risk of bias: -1 Serious -2 Very serious	Large effect +1 Large +2 Very large
	Moderate	Inconsistency -1 Serious -2 Very serious	Dose response +1 Evidence of a gradient
Observational study ⟶	Low	Indirectness -1 Serious -2 Very serious	All plausible confounding +1 Would reduce a demonstrated effect or +1 Would suggest a spurious effect when results show no effect
	Very low	Imprecision -1 Serious -2 Very serious	
		Publication bias -1 Likely -2 Very likely	

*From:
Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction - GRADE evidence profiles and summary of findings tables. Journal of Clinical Epidemiology 2011;64(4):383-94.

Appendix Table 3. Study selection criteria of meta-analyses of intervention studies* on the effect of vitamin D supplementation on cancer outcomes

Meta-analysis	Inclusion criteria	Exclusion criteria
Sperati, 2013 (15)	1) Compared with placebo/no treatment 2) Vitamin D as single agent 3) Combined regimens including supplements & lifestyle modifications if used equally in all groups	Pregnant or lactating women
Bjelakovic, 2014 (16)	1) RCTs, irrespective of blinding, publication, status, or language. 2) Any dose, duration and route of administration 3) Monotherapy or in combination with calcium 4) Concomitant interventions if used equally in all intervention groups	1) Secondary induced osteoporosis (e.g., glucocorticoid-induced osteoporosis, thyroidectomy, primary hyperparathyroidism, chronic kidney disease, liver cirrhosis, Crohn's disease, gastrointestinal bypass surgery) 2) Pregnant or lactating women 3) People with cancer
Bolland, 2014 (17)	Cholecalciferol or ergocalciferol	1) Cluster randomised trials 2) Trials of hydroxylated vitamin D or vitamin D analogues 3) Other interventions only in vitamin D group 4) Trials of fortified dairy products 5) Chronic comorbidity other than osteoporosis or frailty
Keum, 2014 (18)	With or without calcium supplementation	1) Non-English articles 2) Abstracts & unpublished reports
Goulão, 2018 (19)	1) Mean or median age of ≥60 years 2) Follow-up ≤1 year 3) Any vitamin D or vitamin D analog 4) Co-administration of other medications (e.g., calcium) if the comparator group received the same medication 5) All languages	1) Renal impairment, steroid-induced osteoporosis or psoriasis 2) Non-melanoma skin cancers not counted as events
Haykal, 2019 (22)	1) Primary prevention 2) Vitamin D compared to placebo 3) Vitamin D for ≥3 years	
Keum, 2019 (20)	Cholecalciferol or ergocalciferol, with or without other nutrients	1) Number of outcomes ≤10 2) Follow-up ≤1 year

Zhang, 2019 (21)	1) Age ≥18 years 2) Any health conditions 3) Vitamin D (any dose) vs. placebo or no treatment 4) Concomitant agents had to be same dose in all groups	1) Case reports, case series, observational studies 2) All participants received vitamin D 3) Pregnant or lactating women 4) Critically patients 5) Hydroxylated vitamin D or vitamin D analogues

*All were randomized controlled trials.

Appendix Table 4. AMSTAR-2 ratings of meta-analyses of intervention studies on the effect of vitamin D supplementation on cancer outcomes

AMSTAR-2 item		First author, publication year (citation)							
Item	Description	Bolland, 2014 (17)	Sperati, 2013 (15)	Bjelakovic, 2014 (16)	Keum, 2014 (18)	Goulão, 2018 (19)	Haykal, 2019 (22)	Keum, 2019 (20)	Zhang, 2019 (21)
1	Did the research questions and inclusion criteria include the components of PICO?	✓	✓	✓	✓	✓	✓	✓	✓
2*	**Did the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?**	×	✓	✓	×	×	×	×	✓
3	Did the review authors explain their selection of the study designs for inclusion in the review?	×	×	×	✓	×	×	✓	×
4*	**Did the review authors use a comprehensive literature search strategy?**	×	✓	✓	×	✓	Partial ✓	Partial ✓	✓
5	Did the review authors perform study selection in duplicate?	×	✓	✓	✓	×	✓	✓	✓
6	Did the review authors perform data extraction in duplicate?	✓	✓	✓	✓	✓	✓	✓	✓
7*	**Did the review authors provide a list of excluded studies and justify the exclusions?**	✓	✓	✓	×	×	×	×	✓
8	Did the review authors describe the included studies in adequate detail?	✓	✓	✓	✓	✓	✓	✓	Partial ✓
9*	**Did the review authors use a satisfactory technique to assess the RoB in studies that were included in the review?**	×	✓	✓	×	✓	✓	×	✓
10	Did the review authors report on the sources of funding for the studies included in the review?	×	×	✓	×	×	×	×	×
11*	**If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?**	✓	✓	✓	✓	✓	✓	✓	✓
12	If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	×	✓	✓	×	✓	✓	✓	✓
13*	**Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?**	×	✓	✓	×	✓	✓	×	✓
14	Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	✓	✓	✓	✓	✓	✓	✓	✓

15	If they performed quantitative synthesis did the review authors investigate publication bias and discuss its likely impact on the results?	✓	✓	✓	✓	✓	✓	✓	✓
16	Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	✓	✓	✓	✓	✓	✓	✓	✓
	Rating of overall confidence in the results of the review	CL	Moderate	High	CL	CL	CL	CL	Moderate

*Critical domains. CL = critically low; RoB = risk of bias.

Appendix Table 5. Umbrella review assessment of meta-analyses of intervention studies on the effect of vitamin D supplementation on cancer outcomes

Reference	Effect (95% CI)	>1000 events (cases)	Significant summary associations per random-effects calculations			No evidence of small-study effects	No evidence of excess of significance	Prediction intervals excluding null value	Largest study nominally significant (P<0.05)	Not large heterogeneity (I^2<50%)	Umbrella review class
			P<10^{-6}	<10^{-3}	P<0.05						
All cancers											
Bolland, 2014 (17)	RR=0.99 (0.93-1.05)	✓	×	×	×	✓	✓	×	×	✓	NS association
Bjelakovic, 2014 (16)	RR=1.00 (0.94-1.06)	✓	×	×	×	×	✓	×	×	✓	NS association
Keum, 2014 (18)	RR=1.00 (0.94-1.06)	✓	×	×	×	✓	✓	×	×	✓	NS association
Goulão, 2018 (19)	RR=1.03 (0.91-1.15)	✓	×	×	×	✓	✓	×	×	✓	NS association
Haykal, 2019 (22)	RR=0.96 (0.86-1.07)	✓	×	×	×	✓	✓	×	×	✓	NS association
Keum, 2019 (20)	RR=0.98 (0.93-1.03)	✓	×	×	×	✓	✓	×	×	✓	NS association
Breast cancer											
Sperati, 2013 (15)	RR=1.11 (0.74-1.68)	×	×	×	×	✓	✓	×	×	✓	NS association
Bjelakovic, 2014 (16)	RR=0.97 (0.86-1.09)	✓	×	×	×	✓	✓	×	×	✓	NS association
Colorectal cancer											
Bjelakovic, 2014 (16)	RR=1.11 (0.92-1.34)	×	×	×	×	✓	✓	×	×	✓	NS association
Lung cancer											
Bjelakovic, 2014 (16)	RR=0.86 (0.69-1.07)	×	×	×	×	✓	✓	×	×	✓	NS association
Pancreatic cancer											
Bjelakovic, 2014 (16)	RR=0.91 (0.57-1.46)	×	×	×	×	✓	✓	×	×	✓	NS association
Total cancer mortality											
Bjelakovic, 2014 (16)	RR=0.88 (0.78-0.98)	✓	×	×	✓	×	✓	✓	×	✓	Weak (Class IV)
Keum, 2014 (18)	RR=0.88 (0.78-0.98)	✓	×	×	✓	✓	✓	✓	×	✓	Weak (Class IV)
Goulão, 2018 (19)	RR=0.88 (0.70-1.09)	×	×	×	×	✓	✓	×	×	✓	NS association
Haykal, 2019 (22)	RR=0.87 (0.79-1.06)	×	×	×	✓	✓	✓	✓	×	✓	Weak (Class IV)

Keum, 2019 (20)	RR=0.87 (0.79-0.96)	✓	×	×	✓	✓	✓	✓	×	✓	Weak (Class IV)
Zhang, 2019 (21)	RR=0.84 (0.74-0.95)	×	×	×	✓	✓	✓	✓	×	✓	Weak (Class IV)

NS = non-significant; RCT = randomized controlled trial; RR = relative risk.

Appendix Table 6. GRADE summary of findings for meta-analyses of intervention studies on the effect of vitamin D supplementation on cancer outcomes

Reference	Studies (n)	Study design	Risk of bias	Imprecision	Inconsistency	Indirectness	Publication bias	Effect (95% CI)	Certainty (GRADE)
All cancers									
Bolland, 2014 (17)	7	RCT	Not serious	Not serious	Not serious	Not serious	Possible: Egger's P-value=0.05	RR=0.99 (0.93-1.05)	+++ Moderate
Bjelakovic, 2014 (16)	14	RCT	Not serious	Not serious	Not serious	Not serious	Likely: Egger's P-value=0.007	RR=1.00 (0.94-1.06)	+++ Moderate
Keum, 2014 (18)	4	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=1.00 (0.94-1.06)	++++ High
Goulão, 2018 (19)	24	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=1.03 (0.91-1.15)	++++ High
Haykal, 2019 (22)	9	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.96 (0.86-1.07)	++++ High
Keum, 2019 (20)	10	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.98 (0.93-1.03)	++++ High
Breast cancer									
Sperati, 2013 (15)	2	RCT	Not serious	Serious: wide CI from benefit to appreciable harm	Not serious	Not serious	Unlikely	RR=1.11 (0.74-1.68)	++ Low
Bjelakovic, 2014 (16)	7	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.97 (0.86-1.09)	+++ Moderate
Lung cancer									
Bjelakovic, 2014 (16)	5	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.86 (0.69-1.07)	+++ Moderate
Colorectal cancer									
Bjelakovic, 2014 (16)	5	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=1.11 (0.92-1.34)	+++ Moderate
Pancreatic cancer									
Bjelakovic, 2014 (16)	2	RCT	Not serious	Serious: wide CI from appreciable benefit to appreciable harm	Not serious	Not serious	Too few studies to assess	RR=0.91 (0.57-1.46)	+++ Moderate
Total cancer mortality									
Bjelakovic, 2014 (16)	4	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.88 (0.78-0.98)	++++ High
Keum, 2014 (18)	3	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.88 (0.78-0.98)	++++ High

Goulão, 2018 (19)	7	RCT	Not serious	Serious: wide CI from appreciable benefit to small harm	Not serious	Not serious	Unlikely	RR=0.88 (0.70-1.09)	+++ Moderate
Haykal, 2019 (22)	5	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.87 (0.79-0.96)	++++ High
Keum, 2019 (20)	5	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.87 (0.79-0.96)	++++ High
Zhang, 2019 (21)	12	RCT	Not serious	Not serious	Not serious	Not serious	Unlikely	RR=0.84 (0.74-0.95)	++++ High

CI = confidence interval; RCT=randomized controlled trial; RR=relative risk.