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Article

Circulating Vitamin D and Colorectal Cancer Risk: An International Pooling Project of 17 Cohorts

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Abstract

Background: Experimental and epidemiological studies suggest a protective role for vitamin D in colorectal carcinogenesis, but evidence is inconclusive. Circulating 25-hydroxyvitamin D [25(OH)D] concentrations that minimize risk are unknown. Current Institute of Medicine (IOM) vitamin D guidance is based solely on bone health.

Methods: We pooled participant-level data from 17 cohorts, comprising 5,706 colorectal cancer cases and 7,107 controls with a wide range of circulating 25(OH)D concentrations. For 30.1% of participants, 25(OH)D was newly measured. Previously measured 25(OH)D was calibrated to the same assay to permit estimating risk by absolute concentrations. Study-specific relative risks (RR) for prediagnostic season-standardized 25(OH)D concentrations were calculated using conditional logistic regression and pooled using random-effects models.

Results: Compared with the lower range of sufficiency for bone health (50-<62.5 nmol/L), deficient 25(OH)D (<30 nmol/L) was associated with 31% higher colorectal cancer risk (RR=1.31, 95% CI=1.05 to 1.62). 25(OH)D above sufficiency (75-<87.5 and 87.5-<100 nmol/L) was associated with 19% (RR=0.81, 95% CI=0.67 to 0.99) and 27% (RR=0.73, 95% CI= 0.59 to 0.91) lower risk, respectively. At 25(OH)D≥100 nmol/L, risk did not continue to decline, and was not statistically significantly reduced (RR=0.91, 0.67 to 1.24, 3.5% of controls). Associations were minimally affected when adjusting for body mass index, physical activity, or other risk factors. For each 25 nmol/L increment in circulating 25(OH)D, colorectal cancer risk was 19% lower in women (RR=0.81, 95% CI=0.75 to 0.87) and 7% lower in men (RR=0.93, 95% CI=0.86 to 1.00) (two-sided $P_{\text{heterogeneity by sex}}$=.008). Associations were inverse in all subgroups, including colorectal subsite, geographic region, and season of blood collection.

Conclusions: Higher circulating 25(OH)D was related to a statistically significant, substantially lower colorectal cancer risk in women and non-statistically significant lower risk in men. Optimal

25(OH)D concentrations for colorectal cancer risk reduction, 75-100 nmol/L, appear higher than current IOM recommendations.

Abbreviations: 25-hydroxyvitamin D (25(OH)D), Confidence Interval (CI), Institute of Medicine (IOM) National Institute of Standards and Technology (NIST), Randomized Clinical Trials (RCTs), Relative Risk (RR), Vitamin D Receptor (VDR)

INTRODUCTION

Vitamin D, obtained through sun exposure, natural and fortified foods, and dietary supplements, is hypothesized to lower colorectal cancer risk *via* anti-proliferative, pro-apoptotic, and anti-angiogenic properties (1). Many prospective cohort studies have reported non-statistically significant lower risk of colorectal cancer with higher prediagnostic concentrations of 25-hydroxyvitamin D [25(OH)D], the accepted measure of vitamin D status (2). However, because individual studies used different assays and laboratories, and vitamin D concentrations differ noticeably across assay methods (3), these studies, and meta-analyses that combine them, are unable to explore the vitamin D-colorectal cancer relationship on the same absolute scale. Further, individual studies have had limited power to examine associations by sex and other population characteristics. The relatively few randomized clinical trials (RCTs) of vitamin D supplementation and colorectal cancer or adenoma have not demonstrated statistically significant effects (5, 6); but study size, duration and timing of supplementation, and compliance may have contributed to the null findings (5, 7). In addition, the RCTs could not evaluate a wide range of vitamin D exposures.

In 2011, the Institute of Medicine (IOM; now the National Academy of Medicine) concluded that evidence of vitamin D benefits for cancer was insufficient to inform Dietary Reference Intakes for vitamin D and more research was needed. Of the cancers, the most evidence for a protective role for vitamin D existed for colorectal cancer, but IOM experts stated that the dose-response relationship was speculative. Therefore, IOM based its dietary intake recommendations and suggestions for circulating 25(OH)D concentrations solely on bone health research (8).

To leverage the variation in circulating 25(OH)D across populations and to identify optimal concentrations for colorectal cancer risk reduction, we examined the association between circulating 25(OH)D and subsequent colorectal cancer incidence in 17 prospective cohorts participating in the international Circulating Biomarkers and Breast and Colorectal Cancer Consortium. We harmonized and pooled participant-level data and based all 25(OH)D measures on

a single, widely accepted assay and laboratory. This approach facilitated the examination of colorectal cancer risk over a wide range of absolute 25(OH)D blood concentrations and permitted comparison of our findings to public health recommendations.

METHODS

Study Design

Seventeen cohorts participated in this pooling project (**Table 1**) (9-25). Prospective studies were eligible for inclusion if they had prediagnostic 25(OH)D data or stored prediagnostic blood samples for at least 50 male or 50 female colorectal cancer cases. Controls were selected using incidence density sampling and matched on sex, age, date of blood draw, and other study-specific factors (see **Supplementary Methods**); most were matched one control per case. Men and women in the same cohort were analyzed separately. Each cohort and the consortium received approval from its respective institutional review board.

25(OH)D Assays

For the eight cohorts without circulating 25(OH)D data (30.1% of participants), 25(OH)D was measured for cases and controls at Heartland Assays, LLC (Ames, IA) using a direct, competitive chemiluminescence immunoassay. Laboratory personnel were blinded to case-control status. For the other nine cohorts, which had previously assayed 25(OH)D, 25(OH)D measurements were calibrated to the Heartland Assays immunoassay. For each of these studies, approximately three controls were selected within each decile of the 25(OH)D distribution, re-assayed at Heartland Assays, and used to calibrate the previously measured 25(OH)D data for the study using robust linear regression (26)(see Supplementary Methods). Each assay batch included blinded quality control samples from individual studies and National Institute of Standards and Technology (NIST)

standard reference materials. Coefficients of variation, including within- and between-batch variability, were 5-13% for the study-specific quality controls and 16%, 9%, and 9%, respectively, for the NIST reference materials at 17.7, 32.3, and 49.8 nmol/L.

Data Collection

Each cohort provided participant-level data on demographic, lifestyle and medical risk factors, ascertained close to the time of blood collection and prior to diagnosis, and for cases, tumor subsite and stage. All variables were harmonized centrally to create uniform definitions across studies.

Outcome

First primary colorectal cancers (International Classification of Diseases Oncology codes: C18.0, C18.2–C18.9) were ascertained via medical or health insurance record review, linkage with population-based cancer registries, self-report, and/or follow-up with physicians or next-of-kin.

Statistical Analysis

To adjust for seasonal variation in circulating 25(OH)D, for each study we regressed 25(OH)D concentrations in controls on week of blood draw using sine-cosine functions (27). A season-standardized value for each participant was calculated by adding the participant's residual from the regression to the study-specific predicted mean in controls (regression intercept). This value represents the participant's circulating 25(OH)D averaged over the entire year according to the seasonal variation observed in the individual study. All reported 25(OH)D values are season-standardized unless otherwise noted.

Circulating 25(OH)D concentrations were categorized using consortium-wide sex-specific quintiles based on the distribution in controls, or IOM-suggested cut-points (8). Restricted cubic spline analyses using aggregated models, which combined all studies into a single dataset and

analyzed them together while controlling for study, indicated that the 25(OH)D-colorectal cancer association was consistent with linearity (*P*>.05 for non-linearity). Therefore, we also modeled circulating 25(OH)D as a continuous variable.

A two-stage approach was used to calculate pooled estimates: study-specific relative risks (RR) were first estimated with conditional logistic regression using age- and time-matched odds ratios (28) and then pooled using random-effects models (29). Model 1 was conditioned on study-specific matching factors. Model 2 also included BMI and physical activity. The fully adjusted model (Model 3) additionally included established and suspected colorectal cancer risk factors (See **Supplementary Methods**). Dietary factors, including calcium, fiber, folate, and red and processed meat, were considered but had negligible impact on results and were therefore not included in the final model.

Heterogeneity among studies was evaluated using the Q statistic (29, 30). Wald statistics for trend used the median 25(OH)D of each category. Effect modification by colorectal cancer risk factors, season of blood draw, geography, and time between blood draw and diagnosis was evaluated using meta-regression (31); differences by cancer subsite were assessed using a contrast test (32). Additional study methods are provided in **Supplementary Methods**. All *P* values were based on two-sided tests and considered statistically significant if *P*<.05. Analyses were conducted using SAS version 9.3 (Cary, NC).

RESULTS

This analysis included 5,706 colorectal cancer cases and 7,107 controls from prospective cohorts in the U.S. (n=11), Europe (n=5), and Asia (n=1) (**Table 1**). Overall, 50.6% of participants were women and most were white (83.9%). Median (10^{th}-90^{th} percentile) age at blood draw was 60 (48-72) years, and median time from blood draw to diagnosis was 6 (1-15) years. Median circulating 25(OH)D in controls, after calibration and season-standardization, was 56 (31-85)

nmol/L overall, 54 (29-83) nmol/L in women, and 58 (34-86) nmol/L in men. Among the cohorts that had previously measured 25(OH)D, calibrated, season-standardized medians ranged from 27.6% lower to 41.9% higher than the original 25(OH)D.

Since 25(OH)D measurements from each study were based on the same assay, we created consortium-wide sex-specific quintiles to take advantage of the wide range of circulating 25(OH)D across studies. We used the third quintile as the referent to avoid excluding the one study with no cases in the lowest quintile in the pooled analyses (**Table 2**). In a conditional logistic regression model with no added covariates (Model 1), participants in the lowest 25(OH)D quintile had a 23% higher risk of colorectal cancer (RR=1.23, 95% CI=1.06 to 1.43) and those in the highest quintile had a 21% lower risk (RR=0.79, 95% CI=0.68 to 0.92) (quintile cut-points provided in **Table 2**). Adjustment for BMI and physical activity (Model 2) minimally impacted the risk estimates. In the model including all recognized non-dietary colorectal cancer risk factors plus alcohol consumption (Model 3), these associations persisted. Participants in quintiles 1 and 5 had 15% higher (RR=1.15, 95% CI=0.97 to 1.38) and 22% lower risks (RR=0.78, 95% CI=0.65 to 0.94), respectively, than quintile 3 (P_{trend}<.001). Excluding one study at a time revealed that no individual study substantially influenced the results (data not shown). The inverse trend was statistically significant in women (p_{trend}<0.001) and weaker and not statistically significant in men (P_{trend}=0.20). The difference by sex was most apparent at high 25(OH)D concentrations ($P_{heterogeneity\ by\ sex}$=.72 and .02 in 1st and 5th quintiles, respectively).

Sensitivity analyses with finer adjustment for both physical activity (included only the 11 studies with sufficiently detailed information) and BMI minimally changed the pooled RRs (data not shown). After excluding the first two years of follow-up (18.7% of participants) to evaluate the potential influence of preclinical disease, results were similar (17% higher risk for quintile 1; 20% lower risk for quintile 5; data not shown).

Results were similar in two-stage analyses using random effects and fixed effects models and in aggregated analyses (Supplementary Table 1). Aggregated analyses permitted directly comparing extreme quintiles since all studies, even those not contributing cases to the referent group, could be included. The pooled RR comparing quintile 5 to 1 from aggregated analyses was 0.71 (95% CI=0.62 to 0.81) (**Supplementary Table 1**).

When circulating 25(OH)D concentrations were categorized using absolute cut-points based on those suggested by IOM for bone health (8), control participants were distributed as follows: 25(OH)D<30 nmol/L: 8.8%; 30-<40 nmol/L: 13.4%; 40-<50 nmol/L:16.5%; 50-<62.5 nmol/L: 23.2%; 62.5-<75 nmol/L: 18.9%; 75-<87.5 nmol/L: 10.5%; 87.5-<100 nmol/L: 5.0%; and ≥100 nmol/L: 3.5%. A statistically significant 31% higher risk (RR=1.31, 95% CI=1.05 to 1.62, **Figure 1**, **Table 3**) was observed at 25(OH)D concentrations <30 nmol/L, levels considered deficient, compared to 50-62 nmol/L, the lower range of sufficiency (IOM defines sufficiency as 50-<75 nmol/L (8)). Circulating 25(OH)D concentrations of 75-<87.5 and 87.5-<100 nmol/L, ranges considered beyond sufficiency, were associated with statistically significant 19% (RR=0.81, 95% CI=0.67 to 0.99) and 27% (RR=0.73, 95% CI=0.59 to 0.91) lower risks of colorectal cancer, respectively. For 75-<100 nmol/L 25(OH)D, risk was 22% lower (RR=0.78, 95% CI=0.67 to 0.92). However, at concentrations ≥100 nmol/L, risk did not continue to decline (RR=0.91, 95% CI=0.67 to 1.24, 3.5% of controls).

In continuous models, each 25 nmol/L increase in circulating 25(OH)D was associated with a statistically significant lower risk of colorectal cancer in women and men combined (RR=0.87, 95% CI=0.82 to 0.92) and in women (RR=0.81, 95% CI=0.75 to 0.87), and with a non-statistically significant lower risk in men (RR=0.93, 95% CI=0.86 to 1.00) (**Table 2**, $P_{\text{heterogeneity by sex}}$=.008).

Nearly all of the individual study RRs for a 25 nmol/L increment in 25(OH)D were inverse, but only five reached statistical significance (**Figure 2**) while the pooled RR was highly statistically significant (P=3.4 x 10^{-7}). Associations per a 25 nmol/L increase in 25(OH)D were not statistically

significantly different across subpopulations defined by demographic and lifestyle factors, season of blood collection, geographical location, or time from blood draw to diagnosis (**Figure 3**). Similar statistically significant inverse associations were observed for colon and rectal cancer. Associations appeared stronger for proximal compared to distal colon cancer but results were not statistically significantly different (**Figure 3**, also **Supplementary Table 2**).

DISCUSSION

In this international collaborative analysis of participant-level data for 5,706 colorectal cancer cases and 7,107 controls, colorectal cancer risk decreased steadily and statistically significantly with increasing prediagnostic circulating 25(OH)D up to 100 nmol/L. Circulating 25(OH)D <30 nmol/L, considered deficient for bone health by the IOM, was associated with a 31% greater risk of colorectal cancer compared with 50-<62.5 nmol/L, the lower range of 25(OH)D considered sufficient for bone health (8). Colorectal cancer risk was lower at 25(OH)D concentrations above those considered sufficient for bone health: 19% and 27% lower risk for 75-<87.5 and 87.5-<100 nmol/L, respectively. At 25(OH)D concentrations ≥100 nmol/L, risk did not continue to decline. However, 25(OH)D concentrations ≥100 nmol/L were observed in only 3.5% of controls; thus risk estimates for high 25(OH)D were imprecise. In continuous models, RRs below 1.0 were observed in all subpopulations examined, including those defined by sex, colorectal subsite, geographic region, and season of blood collection. However, there was a statistically significant interaction by sex whereby the inverse association in women was stronger than that in men, most notably at higher circulating 25(OH)D.

Previous meta-analyses of prospective cohorts have reported statistically significant reductions in colorectal cancer risk for “high” vs. “low” 25(OH)D (22, 33), but have not taken into account the high variability in 25(OH)D measurements across assays and laboratories used in the

included studies (3). In 2017, the World Cancer Research Fund/American Institute for Cancer Research Continuous Update Project , which reviewed data from 11 cohorts with prediagnostic circulating 25(OH)D, considered evidence for a role of vitamin D in colorectal carcinogenesis to be "limited" (2). Our consortium chose a single, widely accepted 25(OH)D assay for all new measurements, calibrated old measurements to the chosen assay, and adjusted for seasonal variation in vitamin D levels using a standardized approach across studies. This approach, still rare in pooled analyses of circulating biomarkers, enabled us to 1) generate exposure-risk relationships over a wide 25(OH)D range and 2) evaluate risk by 25(OH)D concentrations relevant to public policy. In addition, our standardized approach to harmonizing and analyzing primary participant-level data from all cohorts allowed us to control for confounding and examine risk subgroups and tumor subtypes uniformly, something not possible in meta-analyses.

At its initiation, this consortium included nearly all published prospective studies on 25(OH)D and colorectal cancer risk (nine cohorts), and then expanded the study population by 43.0% by assaying 25(OH)D in eight additional cohorts (9-25). Four studies published after initiation of our project (34-37) reported inverse associations, consistent with our findings. Each study included ≤225 cases, and risk estimates were non-statistically significant. Similarly, results for continuous 25(OH)D from all but five of the individual studies participating in our consortium did not reach statistical significance. However, the pooled result was highly statistically significant (P=3.4 x 10^{-7}), demonstrating the value of pooling data.

The relatively few RCTs of supplemental vitamin D and colorectal cancer or adenoma have not demonstrated statistically significant effects. In the largest RCT, the Women's Health Initiative (WHI), 400 IU vitamin D and 1,000 mg calcium/day did not lower colorectal cancer risk in postmenopausal women (5). However, low dose, limited adherence and absence of pre- and post-intervention 25(OH)D data for all participants complicates interpretation of results (7). In addition, only 322 colorectal cancers were diagnosed during the trial. Other RCTs of vitamin D

supplementation had smaller numbers of colorectal cancer outcomes (2 to 158)(38). In a recent RCT, colorectal adenoma recurrence was not reduced by 1000 IU/day of vitamin D (with or without 1200 mg/day calcium) after 3-5 years (6). Five RCTs of high supplemental vitamin D doses (≥2000 IU/day) in men and women are currently ongoing (39). We anticipate our current analysis, with precise estimates for colorectal cancer risk over a wide range of circulating 25(OH)D, will inform the interpretation of RCTs by suggesting the 25(OH)D concentrations at which colorectal cancer incidence is likely reduced.

Although an inverse association between circulating 25(OH)D and colorectal cancer risk was noted in both women and men, a provocative finding of this research is the stronger association observed in women, particularly at high 25(OH)D concentrations. The difference by sex was evident in the majority of the cohorts that included both sexes. Earlier prospective observational studies hinted at a more pronounced association in women than men, but evidence was too limited to draw firm conclusions (4). The strength of some other colorectal cancer risk factors, such as obesity-related factors, has been found to differ by sex (40). Previous reports suggested estrogen might influence vitamin D activity (41, 42), and a reanalysis of the WHI RCT indicated that supplemental vitamin D and calcium was associated with lower colorectal cancer risk only among women not randomized to receive exogenous estrogen (43). However, we observed no effect modification by menopausal hormone therapy or menopausal status at time of blood draw among women in our study, although the number of premenopausal participants was limited. The biological explanation for the more pronounced inverse association in women at high 25(OH)D concentrations that we observed is unclear, and merits additional laboratory and epidemiologic research.

Vitamin D is best known for its critical role in regulating calcium homeostasis and bone mineral metabolism (8), but strong mechanistic evidence supports its importance in colorectal carcinogenesis (1, 44). Experimental (45) and pilot trial data (44) report anti-proliferative, pro-

differentiation, and pro-apoptotic effects of vitamin D. The nuclear vitamin D receptor (VDR), present in many tissues including the large bowel, influences expression of 3-5% of the human genome (1), including many genes involved in cell cycle regulation. Circulating 25(OH)D is the precursor to the active form of vitamin D ($1,25(OH)_2D$), which binds to VDR to modulate gene transcription. Local synthesis and degradation of $1,25(OH)_2D$ by the enzymes CYP27B1 and CYP24A1, respectively, occurs in an autocrine/paracrine fashion in many tissues, including colorectal mucosa. In human rectal mucosa, vitamin D supplementation upregulates *CYP27B1* and *CYP24A1* expression and modulates expression of the adenomatous polyposis coli gene (*APC*) and cell cycle regulation pathways (44). Additional potential mechanisms for vitamin D activity specific to the large bowel include promoting detoxification of DNA-damaging lithocholic acid (46) and improving gut mucosal integrity (47) and immunity (48).

Strengths of this study include harmonization of participant-level data on exposures and outcomes from 17 cohorts and analysis with a common statistical approach, thereby removing the potential heterogeneity present in meta-analyses of the published literature. The vast majority of the prospective data on circulating 25(OH)D and colorectal cancer available worldwide has been included, markedly reducing the potential for publication bias. Our calibration of previously collected 25(OH)D measurements to the same widely accepted assay and laboratory used for the new measurements enabled us to control for differences in assay accuracy across studies and examine risk on the same absolute scale. Our approach permitted a more comprehensive examination of vitamin D-colorectal cancer relationships in population subgroups, by tumor subsites, and across the range of 25(OH)D concentrations than possible in individual studies or meta-analyses. Only studies that collected blood samples prior to colorectal cancer diagnosis were included, which reduced the possibility of disease altering circulating 25(OH)D.

We have also considered potential limitations of our study. Both obesity and physical inactivity are independent colorectal cancer risk factors and inversely correlated with circulating

25(OH)D (49). However, adjustment for both variables, even using finer categorizations available for a subset of studies, had minimal impact on our results. Further, adjustment for established colorectal cancer risk factors, including dietary factors, did not attenuate risk estimates. Minimal confounding by accepted risk factors adds to our confidence in the validity of our findings and strengthens the evidence for causality. Our results are based on a single blood draw, at a median of 5.5 years before diagnosis, which may be an imperfect measure of long-term 25(OH)D status. However, within-individual correlation coefficients for repeat measures of circulating 25(OH)D 1-11 years apart are 0.53-0.81, indicating that a single blood sample can provide relatively stable estimates (21, 50). Finally, despite the large size of the consortium, we had limited power to examine associations at 25(OH)D concentrations >100 nmol/L and in some racial/ethnic subgroups. Future research should evaluate associations at very high 25(OH)D levels, as these levels may now be more common (39), and in subgroups not well represented in our cohorts.

In summary, by demonstrating a strong, statistically significant, and robust inverse association between prediagnostic circulating vitamin D and colorectal cancer risk, we substantially strengthen the evidence, previously considered inconclusive, for a causal relationship. Our study estimates risk over a wide range of absolute 25(OH)D concentrations, a research gap noted by the IOM experts. By clarifying at what 25(OH)D concentrations an effect on colorectal cancer incidence might be observed, our results facilitate interpretation of RCTs of supplemental vitamin D. Finally, our study suggests that optimal circulating 25(OH)D concentrations for colorectal cancer risk reduction are 75–100 nmol/L, higher than current IOM recommendations for bone health. While our results are relevant to future recommendations for optimal vitamin D status, the effects of vitamin D on health outcomes other than colorectal cancer also need to be evaluated and integrated into public health guidance.

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Table 1. Descriptive Characteristics of Participating Cohorts

Cohort	Country or Continent	% Female	Colorectal cancer Cases/ Controls (n)	Median Calibrated and Season-standardized 25(OH)D in Controls, nmol/L (10-90%)[*]	Median Original 25(OH)D in Controls, nmol/L (10-90%)[*]	Median Age at Blood Draw, in years (10-90%)[†]	Years of Blood Draw[†]	Median Years to Diagnosis (10-90%)
Outside U.S.								
Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC1 and ATBC2)[‡,§]	Finland	0	129/250	47 (29-79)	34 (19-62)	59 (52-67)	1985-1988	3.5 (0.9-6.3)
			369/369	44 (27-74)	31 (14-61)	57 (51-65)		12.5 (7.7-17.2)
Breakthrough Generations Study (BGS)[‖]	U.K.	100	92/92	50 (23-82)	48 (23-85)	60 (47-74)	2004-2009	2.3 (0.8-4.6)
European Investigation into Cancer and Nutrition (EPIC)[§]	Europe	49.8	1223/1223	42 (27-63)	58 (32-98)	59 (50-67)	1992-1999	3.6 (0.9-6.7)
JANUS Serum Bank (JANUS)[§]	Norway	51.1	380/380	65 (41-95)	60 (37-89)	44 (40-61)	1972-2002	15.6 (5.1-25.4)
Japan Public Health Center-based Prospective Study (JPHC)[§]	Japan	48.6	367/733	64 (53-78)	62 (43-87)	58 (47-67)	1990-1995	5.1 (1.1-10.2)
Hormones and Diet in the Etiology of Breast Cancer (ORDET) [‖]	Italy	100	130/130	33 (18-61)	30 (13-66)	53 (42-65)	1987-1992	10.8 (3.8-16.0)
U.S.								
Beta-Carotene and Retinol Efficacy Trial (CARET)[‖,¶]	U.S.	0	123/123	52 (33-86)	53 (27-86)	64 (54-69)	1987-1996	4.9 (1.0-10.7)
CLUE II: Campaign Against Cancer and Heart Disease (CLUE II)[‖]	U.S.	54.9	288/288	49 (19-87)	63 (34-102)	62 (46-75)	1989	9.0 (1.8-17.3)
Cancer Prevention Study-II (CPS-II)[‖]	U.S.	51.0	288/288	63 (35-91)	64 (36-92)	72 (64-79)	1998-2001	3.2 (0.7-6.2)
Health Professionals Follow-up Study (HPFS)[§]	U.S.	0	267/517	71 (47-94)	72 (45-99)	68 (53-75)	1993-1995	6.3 (1.4-11.4)
Multiethnic Cohort Study (MEC)[§]	U.S.	35.4	183/346	64 (36-95)	60 (30-96)	69 (57-79)	1995-2006	1.5 (0.3-3.3)
Nurses' Health Study (NHS)[§]	U.S.	100	348/694	64 (41-95)	60 (38-87)	60 (49-67)	1989-1991	9.6 (2.1-16.2)
New York University Women's Health Study (NYU WHS)[‖]	U.S.	100	235/235	55 (34-91)	57 (30-89)	58 (44-64)	1985-1991	12.3 (2.5-18.9)
Physicians' Health Study (PHS)[§]	U.S.	0	224/379	61 (43-88)	61 (35-97)	57 (46-68)	1982-1983	9.5 (2.6-15.4)
Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO)[‖]	U.S.	43.3	473/473	55 (29-87)	56 (29-86)	65 (58-72)	1993-2001	5.4 (0.2-11.3)
Women's Health Initiative (WHI)[§]	U.S.	100	290/290	50 (33-80)	44 (18-86)	67 (57-75)	1994-2009	3.2 (0.6-6.5)

Women's Health Study (WHS)[‖]	U.S.	100	297/297	54 (31-89)	57(32-91)	58 (48-69)	1993-1995	8.0 (2.3-13.4)
Total		50.6%	5706/7107	56 (31-85)	58 (29-91)	60 (48-72)	1972-2009	5.5 (1.1-15.0)

Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; U.K., United Kingdom; U.S., United States.
SI conversion factors: To convert 25(OH)D to ng/mL, divide values by 2.496.
[*]Newly measured or calibrated, season-standardized circulating 25(OH)D.
[†]In cases and controls combined.
[‡]Data from the two publications from the ATBC cohort were analyzed as separate studies because the cases were from different, non-overlapping follow-up periods, and 25(OH)D assays were conducted at different laboratories and times.
[§]Calibration studies: For each study that had previously measured circulating 25(OH)D, approximately three samples were selected from within each decile of the 25(OH)D distribution in the control participants (n=29 samples) in that study and re-assayed during 2011-2013 using the same assay and laboratory used to measure 25(OH)D concentrations in the new studies. These results were then used to calibrate the previously measured 25(OH)D data for all cases and controls in that study to the assay used in the new studies.
[‖]New studies: Circulating 25(OH)D was analyzed with a direct, competitive chemiluminescence immunoassay at Heartland Assays, Ames IA from November 2011 through April 2013.
[¶]Only men in the CARET study were included in the analyses due to too few cases occurring in women (n<50).

Table 2. Pooled Associations of Consortium-Wide, Sex-Specific Quintiles and Continuous Concentrations of 25(OH)D with Colorectal Cancer

Model	Consortium-Wide, Sex-Specific Quintiles of Circulating 25(OH)D[*]								Per 25 nmol/L of Circulating 25(OH)D[*]		
	RR (95% CI)					*P* Trend[‡]	*P* Het by Study[§]	*P* Het by Sex[‖]	RR (95% CI)	*P* Het by Study[§]	*P* Het by Sex[‖]
	Q1	Q2	Q3[†]	Q4	Q5						
Overall											
Cases/controls	1519/1421	1247/1421	1094/1423	957/1421	889/1421						
Model 1[¶]	1.23 (1.06 to 1.43)	1.02 (0.89 to 1.18)	1.00	0.91 (0.80 to 1.04)	0.79 (0.68 to 0.92)	<.001	.17, .12	.50, .02	0.85 (0.80 to 0.89)	.28	.01
Model 2[#]	1.21 (1.02 to 1.42)	1.01 (0.87 to 1.17)	1.00	0.93 (0.82 to 1.07)	0.80 (0.68 to 0.95)	<.001	.09, .06	.62, .01	0.86 (0.81 to 0.91)	.20	.01
Model 3[**]	1.15 (0.97 to 1.38)	0.98 (0.83 to 1.16)	1.00	0.92 (0.79 to 1.07)	0.78 (0.65 to 0.94)	<.001	.09, .06	.72, .02	0.87 (0.82 to 0.92)	.41	.008
Women											
25(OH)D (nmol/L)	< 37	37- <49	49- <59	59- <72	≥ 72						
Cases/controls	822/706	654/705	574/707	476/706	422/705						
Model 1[¶]	1.33 (1.11 to 1.59)	1.02 (0.84 to 1.24)	1.00	0.85 (0.71 to 1.01)	0.67 (0.56 to 0.81)	<.001	.43, .98		0.80 (0.74 to 0.85)	.89	
Model 2[#]	1.29 (1.06 to 1.57)	1.02 (0.85 to 1.23)	1.00	0.87 (0.72 to 1.04)	0.67 (0.56 to 0.82)	<.001	.37, .94		0.80 (0.75 to 0.86)	.92	
Model 3[**]	1.21 (0.97 to 1.52)	1.00 (0.82 to 1.23)	1.00	0.83 (0.66 to 1.05)	0.67 (0.54 to 0.81)	<.001	.27, .81		0.81 (0.75 to 0.87)	.82	
Men											
25(OH)D (nmol/L)	< 41	41-<53	53- <63	63- <76	≥ 76						
Cases/controls	697/715	593/716	520/716	481/715	467/716						
Model 1[¶]	1.16 (0.91 to 1.49)	1.03 (0.83 to 1.28)	1.00	0.98 (0.83 to 1.17)	0.91 (0.70 to 1.19)	.02	.09, .02		0.89 (0.82 to 0.97)	.19	
Model 2[#]	1.16 (0.88 to 1.53)	1.01 (0.80 to 1.28)	1.00	1.01 (0.84 to 1.22)	0.95 (0.72 to 1.26)	.13	.04, .02		0.91 (0.83 to 1.00)	.12	
Model 3[**]	1.12 (0.84 to 1.49)	0.98 (0.75 to 1.29)	1.00	1.01 (0.83 to 1.22)	0.93 (0.69 to 1.25)	.20	.06, .02		0.93 (0.86 to 1.00)	.50	

Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; RR, relative risk; 95% CI, 95% confidence interval; Q1-5, quintile; Het, heterogeneity

[*] Consortium-wide, sex-specific quintiles and continuous concentration are based on newly measured and calibrated season-standardized circulating 25(OH)D. Quintile cut-points are based on the distributions in the controls, separately for each sex, as shown.

[†] Quintile 3 was used as the referent category because one cohort (Japan Public Health Center-based Prospective Study), with no cases in consortium-wide quintile 1, would have been dropped from the analysis if quintile 1 was used as the referent category.

[‡] *P*-value, two-sided test for trend calculated using a continuous variable based on the median 25(OH)D in each quintile.

[§] *P*-value, two-sided test for between-studies heterogeneity. Results are for quintile 1 and quintile 5 for quintile-based analyses and for β-coefficient for continuous analyses.

[‖] *P*-value, two-sided test for between-studies heterogeneity due to sex. Results are for quintile 1 and quintile 5 for quintile-based analyses and for β-coefficient for continuous analyses.

[¶]Model 1, conditioned on study-specific matching factors (see **Supplementary Methods**), including date of blood draw and age.

[#]Model 2, Model 1 adjusted for body mass index (<20, 20-<22.5, 22.5-<25, 25-<27.5, 27.5-<30, ≥30 kg/m^2) and physical activity (study-specific tertiles of metabolic equivalents in hours/week, if available, or low, moderate, high).

[**]Model 3, Model 2 additionally adjusted for race (white, black, Asian, other, for studies that did not match on race); family history of colorectal cancer (yes, no); alcohol consumption (men: 0, >0-<5, 5-<15, 15-<30, ≥30 g/d; women: 0, >0-<5, 5-<15, ≥15 g/d); smoking status (never, former, current); aspirin and/or non-steroidal anti-inflammatory drug use (yes, no for regular use, available for 9 cohorts only); and in women, menopausal status and menopausal hormone therapy (postmenopausal/never hormone use, postmenopausal/former hormone use, postmenopausal/current hormone use, postmenopausal/missing hormone use, premenopausal, perimenopausal or missing menopausal status). For the European Prospective Investigation into Cancer and Nutrition, this variable was modeled as postmenopausal/not current hormone use and postmenopausal/current hormone use, and for the Japan Public Health Center-based Prospective Study, this variable was modeled as postmenopausal/never hormone use and postmenopausal/ever hormone use). All covariates included a missing category.

Table 3. Pooled Associations of 25(OH)D for Categories Based on Institute of Medicine Cut-points with Colorectal Cancer[*]

Model	Circulating 25(OH)D, nmol/L RR (95% CI)								*P* Trend[†]	*P* Het by Study[‡]	*P* Het by Sex[§]
	<30	30-<40	40-<50	50-<62.5	62.5-<75	75-<87.5	87.5-<100	≥100			
Overall											
Cases/controls	735/625	908/954	1061/1176	1259/1649	896/1346	464/748	208/357	175/252			
Multivariable RR[‖]	1.31 (1.05 to 1.62)	1.05 (0.88 to 1.24)	1.05 (0.87 to 1.25)	1.00 (ref)	0.94 (0.83 to 1.07)	0.81 (0.67 to 0.99)	0.73 (0.59 to 0.91)	0.91 (0.67 to 1.24)	<.001	.13, .18	.75, .56
Women											
Cases/controls	468/389	499/496	568/593	649/824	410/632	181/318	100/165	73/112			
Multivariable RR[‖]	1.37 (1.08 to 1.75)	1.08 (0.88 to 1.33)	1.09 (0.89 to 1.35)	1.00 (ref)	0.88 (0.73 to 1.05)	0.67 (0.52 to 0.86)	0.67 (0.49 to 0.93)	0.83 (0.53 to 1.31)	<.001	.38, .34	
Men											
Cases/controls	267/236	409/458	493/583	610/825	486/714	283/430	108/192	102/140			
Multivariable RR[‖]	1.28 (0.86 to 1.90)	1.00 (0.75 to 1.35)	1.03 (0.75 to 1.40)	1.00 (ref)	1.00 (0.82 to 1.23)	0.96 (0.69 to 1.33)	0.79 (0.58 to 1.07)	0.95 (0.61 to 1.48)	.12	.07, .14	

Abbreviations: 25(OH)D, 25-Hydroxyvitamin D; RR, relative risk; 95% CI, 95% confidence interval; Het, heterogeneity

[*] Concentrations are based on newly measured and calibrated season-standardized circulating 25(OH)D. Categories are based on Institute of Medicine (IOM) recommendations for bone health: deficiency (<30 nmol/L), insufficiency (30-<40 and 40-<50 nmol/L), sufficiency [50-<62.5 (referent) and 62.5- <75 nmol/L] and beyond sufficiency (75-<87.5, 87.5-<100, and ≥100 nmol/L). RR for 75 to <100 nmol/L vs. 50 to <62.5 nmol/L=0.78 (95% CI, 0.67 to 0.92) overall, =0.67 (95% CI, 0.54 to 0.83) for women and =0.90 (95% CI, 0.68 to 1.18) for men.

[†] *P*-value, two-sided test for trend calculated using a continuous variable based on the median 25(OH)D in each category.

[‡] *P*-value, two-sided test for between-studies heterogeneity. Results are for the lowest and highest category, respectively.

[§] *P*-value, two-sided test for between-studies heterogeneity due to sex. Results are for the lowest and highest category, respectively.

[‖] Multivariable RR (equivalent to Model 3 in Table 2), conditioned on study-specific matching factors (see **Supplementary Methods**), including date of blood draw and age, and additionally adjusted for body mass index (<20, 20-<22.5, 22.5-<25, 25-<27.5, 27.5-<30, ≥30 kg/m^2); physical activity (study-specific tertiles of metabolic equivalents in hours/week, if available, or low, moderate, high); race (white, black, Asian, other, for studies that did not match on race); family history of colorectal cancer (yes, no); alcohol consumption (men: 0, >0-<5, 5-<15, 15-<30, ≥30 g/d; women: 0, >0-<5, 5-<15, ≥15 g/d); smoking status (never, former, current); aspirin and/or non-steroidal anti-inflammatory drug use (yes, no for regular use, available for 9 cohorts only); and in women, menopausal status and menopausal hormone therapy (postmenopausal/never hormone use, postmenopausal/former hormone use, postmenopausal/current hormone use, postmenopausal/missing hormone use, premenopausal, perimenopausal or missing menopausal status). For the European Prospective Investigation into Cancer and Nutrition, this variable was modeled as postmenopausal/not current hormone use and postmenopausal/current hormone use, and for the Japan Public Health Center-based Prospective

Study, this was modeled as postmenopausal/never hormone use and postmenopausal/ever hormone use). All covariates included a missing category.

FIGURE LEGENDS

Figure 1. Pooled multivariable relative risks (RR, indicated by open symbols) and 95% confidence intervals (95% CI, indicated by vertical lines) of colorectal cancer according to categories of season-standardized circulating 25(OH)D concentrations (A) overall, (B) women, and (C) men. 25(OH)D categories correspond to Institute of Medicine (IOM) recommendations for bone health: deficiency defined as <30 nmol/L; insufficiency includes 30-<40 and 40-<50 nmol/L; sufficiency includes 50-<62.5 (referent) and 62.5-<75 nmol/L; and beyond sufficiency includes 75-<87.5, 87.5-<100, and ≥100 nmol/L. The RR (95%CI) for each category is plotted at the median concentration of 25(OH)D among controls in that category. The RRs for 75-<100 nmol/L, relative to 50-<62.5 nmol/L, are 0.78 (95% CI=0.67 to 0.92) overall, 0.67 (95% CI=0.54 to 0.83) for women, and 0.90 (95% CI=0.68 to 1.18) for men (not shown in figure). Models were conditioned on study-specific matching factors, including date of blood draw and age and additionally adjusted for body mass index, physical activity, race, family history of colorectal cancer, alcohol consumption, smoking status, aspirin and/or NSAID use, and, in women, menopausal status and menopausal hormone therapy. See Supplementary Methods for individual studies excluded in specific 25(OH)D categories due to low numbers of subjects. Two-sided *P*-value, test for trend calculated using a continuous variable based on the median 25(OH)D in each category.

Figure 2. Study-specific and pooled multivariable relative risks (RR, indicated by solid squares and diamonds, respectively) and 95% confidence intervals (95% CI, indicated by horizontal lines) of colorectal cancer per 25 nmol/L increment in season-standardized circulating 25(OH)D. The size of the square is proportional to the inverse of the variance of the study-specific RR. Full cohort names

are listed in Table 1. A RR of 1.0, marked by the vertical line, indicates no association. Models were conditioned on study-specific matching factors, including date of blood draw and age, and additionally adjusted for body mass index, physical activity, race, family history of colorectal cancer, alcohol consumption, smoking status, aspirin and/or NSAID use, and, in women, menopausal status and menopausal hormone therapy. Two-sided *P*-value, test for between-studies heterogeneity was calculated using the Q statistic.

Figure 3 Pooled multivariable relative risks (RR, indicated by solid diamonds) and 95% confidence intervals (95% CI, indicated by horizontal lines) of colorectal cancer per 25 nmol/L increment in season-standardized circulating 25(OH)D stratified by demographic, lifestyle, and other factors and tumor characteristics. A RR of 1.0, marked by the vertical line, indicates no association. Summer- and winter-specific RR were not standardized by season. Individual cohorts were excluded from a stratum if there were fewer than 25 cases in that stratum. Conditional models were used for stratified analyses by sex, age at diagnosis, region, 25(OH)D data, tumor stage, colorectal and colon subsite, and time to diagnosis. These models were conditioned on study-specific matching factors, including date of blood draw and age, and additionally adjusted for body mass index, physical activity, race, family history of colorectal cancer, alcohol consumption, smoking status, aspirin and/or NSAID use, and, in women, menopausal status and menopausal hormone therapy, except in models stratified by that variable. Unconditional models, which were used for the remaining stratified analyses, were adjusted for study-specific matching factors including date of blood draw and age, and the covariates listed above. See Supplementary Methods for individual studies excluded in specific strata due to low numbers of subjects. *P*-value, test for between-studies heterogeneity was calculated using the Q statistic except for race. Due to small numbers of non-whites within most individual cohorts, analyses stratified by race were conducted using aggregated

data, adjusted for study. *P*-value, test for heterogeneity across strata, except for race, tumor stage and subsite, was calculated using meta-regression. Statistical significance for interaction by race was assessed using a Wald test. Evaluation of common effects by tumor stage and subsite was assessed using a contrast test. All statistical tests are two-sided.