American Journal of Epidemiology
Vol. 175, No. 7
DOI: 10.1093/aje/kwr350
Advance Access publication:
February 22, 2012

Original Contribution

The Influence of Health and Lifestyle Characteristics on the Relation of Serum 25-Hydroxyvitamin D With Risk of Colorectal and Breast Cancer in Postmenopausal Women

Marian L. Neuhouser*, JoAnn E. Manson, Amy Millen, Mary Pettinger, Karen Margolis, Elizabeth T. Jacobs, James M. Shikany, Mara Vitolins, Lucile Adams-Campbell, Simin Liu, Erin LeBlanc, Karen C. Johnson, and Jean Wactawski-Wende

* Correspondence to Dr. Marian L. Neuhouser, Cancer Prevention Program, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, M4B402, Seattle, WA 98109-1024 (e-mail: mneuhous@fhcrc.org).

Initially submitted May 11, 2011; accepted for publication September 9, 2011.

The authors' objective was to discern whether lifestyle or health-related factors were confounders, effect modifiers, or irrelevant with regard to understanding observational associations of serum 25-hydroxyvitamin D (25(OH)D) with colorectal and breast cancer. The authors conducted nested case-control studies of colorectal cancer (310 cases, 310 controls) and breast cancer (1,080 cases, 1,080 controls) in the Women's Health Initiative Calcium and Vitamin D Clinical Trial (1994–2005). Case-control matching factors included age, latitude, race/ethnicity, and blood collection date. Serum 25(OH)D was assayed in baseline fasting blood. Conditional logistic regression was used to estimate odds ratios for each cancer by serum 25(OH)D concentration, comparing the relative effects of successively adding body mass index, physical activity, and other health and lifestyle characteristics particular to each cancer. In models with matching factors only, low (vs. high) serum 25(OH)D was associated with a colorectal cancer odds ratio of 2.72 (95% confidence interval (CI): 1.55, 4.77) and a breast cancer odds ratio of 1.33 (95% CI: 1.02, 1.72). In multivariate-adjusted models for colorectal cancer, the association strengthened (OR = 4.45, 95% CI: 1.96, 10.10). However, in multivariate-adjusted breast cancer models, associations were no longer significant (OR = 1.06, 95% CI: 0.78, 1.43). Adjusting for health and lifestyle characteristics has differential effects depending on the cancer site; when modeling such relations, investigators should take these factors into account.

breast neoplasms; colorectal neoplasms; 25-hydroxyvitamin D; postmenopausal women; randomized controlled trials

Abbreviations: BMI, body mass index; CaD, Calcium and Vitamin D; CI, confidence interval; 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; MET, metabolic equivalent; WHI, Women's Health Initiative.

The relation between vitamin D and cancer risk is unclear. Support for a potential chemopreventive effect of vitamin D comes from cell lines, animal studies, observational studies, and very small clinical trials (1–7). Nonetheless, the relation of vitamin D to specific cancers, including breast and colorectal cancers, remains controversial, particularly since both clinical trials and observational studies have not been consistent in either the direction or the magnitude of associations (8). The inconsistent findings may be due to: lack of uniformity in identifying the exposure (i.e., serum vitamin D status, self-reports of vitamin D intake from food and supplements, solar exposure); challenges associated with accurately assessing solar exposure (9); use of varying doses in supplement trials; and a lack of understanding about factors that influence biomarkers of vitamin D status (8, 10–12).

In its recent vitamin D consensus report, the Institute of Medicine concluded that there was a lack of evidence in support of a consistent or causal relation between vitamin D and cancer risk and that more research was needed to fill the gaps in understanding vitamin D-cancer associations (13). To understand the role of health and lifestyle characteristics and their influence on serum 25-hydroxyvitamin D (25(OH)D)

Table 1. Demographic, Health, and Lifestyle Characteristics of Participants in a Colorectal Cancer Case-Control Group (310 Cases, 310 Controls), by Serum 25-Hydroxyvitamin Concentration, Women's Health Initiative, 1994–2005[a]

Characteristic	Quartile of Serum 25-Hydroxyvitamin D Concentration, nmol/L												*P* Value
	≥64.5			43.6–<64.5			32.7–<43.6			<32.7			
	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	
Age, years	130		65.6 (64.3)	162		64.9 (6.7)	147		65.1 (7.3)	181		65.1 (6.8)	0.64
Waist circumference, cm	130		84.4 (12.4)	162		87.8 (12.7)	147		98.6 (13.0)	181		92.7 (14.1)	<0.001
Red meat intake, servings/day	129		0.72 (0.53)	158		0.72 (0.58)	145		0.75 (0.50)	177		0.66 (0.44)	0.45
Race/ethnicity													
White	122	93.9		143	88.3		126	85.7		137	75.7		<0.001
Black	1	<1.0		11	6.8		9	6.1		31	17.1		
Hispanic	0	0.00		6	3.7		4	2.7		8	4.4		
Other	7	5.4		2	1.2		8	5.4		5	2.8		
Body mass index[b]													
<25.0	53	40.8		50	30.9		40	27.2		36	19.9		<0.001
25.0–29.9	46	35.4		65	40.1		43	29.3		65	35.9		
30.0–39.9	25	19.2		45	27.8		57	38.8		66	36.5		
≥40.0	6	4.6		2	1.2		7	4.8		14	7.7		
Smoking status													
Never smoker	69	53.5		76	47.5		89	61.0		89	50.0		0.130
Past smoker	52	40.3		73	45.6		43	29.5		76	42.7		
Current smoker	8	6.2		11	6.9		14	9.6		13	7.3		
Physical activity, MET-hours/week													
≤1.5	21	17.8		26	18.3		31	23.7		50	30.9		0.017
≤6.9	30	25.4		43	30.3		29	22.1		50	30.9		
≤16.7	26	22.0		26	18.3		36	27.5		29	17.9		
>16.7	41	34.8		47	33.1		35	26.7		33	20.4		
Total calcium intake, mg/day													
<630	18	13.9		35	22.1		35	24.1		63	35.6		<0.001
630–1,007	22	17.1		41	26.0		40	27.6		51	28.8		
1,008–1,409	42	32.6		37	23.4		38	26.2		35	19.8		
≥1,410	47	36.4		45	28.5		32	22.1		28	15.8		

Table continues

in subsequent development of invasive breast and colorectal cancer, we further analyzed data from 2 case-control studies nested within the Women's Health Initiative (WHI) randomized, placebo-controlled clinical trial of calcium plus vitamin D. The primary trial outcome was hip fracture; other fractures and colorectal cancer were secondary outcomes (14, 15). There was no intervention effect of trial supplementation on the risk of either breast or colorectal cancer (15, 16). These previous reports provided important data on vitamin D-cancer associations, but much remains to be learned about understanding predictors of serum 25(OH)D and how best to model their associations with cancer risk. This report is focused on methodological issues related to understanding the extent to which lifestyle and health-related characteristics influence the association of the principal biomarker of vitamin D, 25(OH)D, with breast and colorectal cancer risk. We expect that the findings will have implications for analytic approaches that should be considered when examining vitamin D-cancer associations.

MATERIALS AND METHODS

Study design

The WHI Clinical Trial was a set of 3 overlapping randomized trials conducted at 40 clinical centers throughout the United States. Details of the trial design, recruitment, and results have been published previously (14, 15). Briefly, eligibility included being a postmenopausal woman aged 50–79 years with a life expectancy of at least 3 years, no prior breast cancer, and no other cancer within the previous 10 years. At baseline (1993–1998), women were enrolled in either the hormone trials ($n = 27,347$) or the dietary modification trial ($n = 48,835$). After 1 year, participants were

Table 1. Continued

Characteristic	Quartile of Serum 25-Hydroxyvitamin D Concentration, nmol/L												*P* Value
	≥64.5			43.6–<64.5			32.7–<43.6			<32.7			
	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	
Alcohol intake													
Nondrinker	15	11.5		10	6.3		14	9.6		31	17.4		0.03
Past drinker	18	13.9		23	14.6		21	14.4		35	19.7		
<1 drink/month	14	10.8		22	13.9		25	17.1		22	12.4		
<1 drink/week	34	26.1		35	22.1		42	28.8		42	23.6		
1–7 drinks/week	36	27.7		42	26.6		24	16.4		33	18.5		
>7 drinks/week	13	10.0		26	16.5		20	13.7		15	8.4		
Total vitamin D intake, IU/day													
<200	27	20.9		51	32.3		50	34.5		97	54.8		<0.001
200–<400	28	21.7		24	15.2		36	24.8		38	21.5		
400–<600	34	26.4		48	30.4		35	24.1		31	17.5		
≥600	40	31.0		35	22.2		24	16.6		11	6.2		
Colorectal cancer screening within past 5 years													
No	54	41.9		68	42.2		67	45.9		84	46.4		0.786
Yes	75	58.1		93	57.8		79	54.1		97	53.6		
Family history of colorectal cancer													
No	104	88.1		124	84.4		115	83.3		132	78.1		0.152
Yes	14	11.9		23	15.7		23	16.7		37	21.9		
Use of hormone therapy at blood draw													
None/past use	54	41.5		79	48.8		78	53.0		115	63.5		0.011
Current use													
Estrogen alone	42	32.3		47	29.0		36	24.5		34	18.8		
Estrogen + progesterone	34	26.1		36	22.2		33	22.4		32	17.7		

Abbreviations: MET, metabolic equivalent; SD, standard deviation.
[a] Cases and controls were matched on age, clinical center location, race/ethnicity, and date of blood collection.
[b] Weight (kg)/height $(m)^2$.

invited to join the WHI Calcium and Vitamin D (CaD) Clinical Trial. These interested and eligible women ($n = 36,282$) were randomized in a double-blind fashion to receive a daily dose of 1,000 mg of elemental calcium plus 400 IU of vitamin D_3 or placebo. Women with a history of hypercalcemia, kidney stones, or corticosteroid or calcitriol use were excluded. Personal use of calcium and vitamin D was permitted during the trial—initially up to 600 IU/day of vitamin D, which was later increased to 1,000 IU/day (17, 18). The protocol was approved by the institutional review boards of each clinical center and the WHI Clinical Coordinating Center. All women provided written informed consent. The trial ended as planned in 2005.

Outcome assessment and case and control definition

Cancer outcomes were identified as part of semiannual health updates. Self-reported cancers were confirmed after review of medical records by trained local and central physician adjudicators (19). During the CaD Trial, 310 colorectal cancers and 1,080 invasive breast cancers were reported and confirmed. In a nested study design, controls were matched to respective cases on age, latitude of the clinical center (or clinical center location if latitude was not available), race/ethnicity, and blood collection date. Breast cancer cases were also matched on WHI trial arm (hormone trials or dietary modification trial) (15, 16).

Blood collection and 25(OH)D assay

Fasting blood samples were collected at year 1 (baseline enrollment in the CaD Trial) using a standard protocol and were stored at −80°C until analysis. Serum concentrations of 25(OH)D were assayed by means of the DiaSorin Liason chemiluminescent assay (DiaSorin, Stillwater, Minnesota). The assay coefficient of variation (11.8%) was determined

Table 2. Demographic, Health, and Lifestyle Characteristics of Participants in a Breast Cancer Case-Control Group (1,080 Cases, 1,080 Controls), by Serum 25-Hydroxyvitamin Concentration, Women's Health Initiative, 1994–2005[a]

Characteristic	Quartile of Serum 25-Hydroxyvitamin D Concentration, nmol/L												*P* Value
	≥64.9			50.9–<64.9			36.7–<50.9			<36.7			
	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	
Age, years	500		62.8 (6.7)	520		62.7 (6.7)	578		63.5 (6.6)	562		62.4 (6.9)	0.032
Waist circumference, cm	500		83.4 (11.8)	520		87.6 (12.5)	578		89.7 (13.1)	563		92.8 (14.2)	<0.001
Gail 5-year risk	500		1.8 (1.0)	520		1.8 (1.0)	578		1.8 (1.1)	562		1.7 (1.0)	0.132
Race/ethnicity													
White	472	94.4		472	90.8		518	89.6		440	78.3		<0.001
Black	10	2.0		17	3.3		34	5.9		85	15.1		
Hispanic	8	1.6		16	3.1		9	1.6		20	3.6		
Other	10	2.0		15	2.9		17	2.9		17	3.0		
Body mass index[b]													
<25.0	207	41.4		155	29.8		152	26.3		87	15.5		<0.001
25.0–29.9	186	37.2		178	34.2		190	32.9		194	34.5		
30.0–39.9	98	19.6		178	34.2		215	37.2		233	41.5		
≥40.0	9	1.8		9	1.7		21	3.6		48	8.5		
Smoking status													
Never smoker	265	53.5		270	52.3		291	50.6		285	51.4		0.090
Past smoker	203	41.0		215	41.7		236	41.0		214	38.6		
Current smoker	27	5.5		31	6.0		48	8.4		56	10.1		
Physical activity, MET-hours/week													
≤1.5	69	15.7		101	21.4		141	27.5		143	28.3		<0.001
≤6.9	95	21.5		115	24.4		120	23.4		158	31.2		
≤16.7	98	22.2		114	24.2		127	24.8		108	21.3		
>16.7	179	40.6		141	29.9		125	24.4		97	19.2		

Table continues

using blinded duplicates in each assay batch. Cases and matched controls were analyzed in the same batch. Laboratory personnel were blinded to the case/control status of samples.

Covariate assessment

Medical history. On average, for women in the CaD Trial, 75%–91% of participants completed mammograms in years 2, 4, 6, and 8 as part of their WHI participation. Colorectal cancer screening was not required as part of trial participation; however, self-reported screening was routine and did not differ between active and placebo women (15). Family history of breast and colorectal cancer was ascertained by self-report. Use of estrogen or estrogen plus progestin was determined by the randomization assignment for hormone trial participants. Since personal use of hormone therapy was permitted for dietary modification trial participants, hormone use by those women was determined by medication inventory for current users and by self-report for those who were not current users. Gail risk scores were calculated from self-reports of personal risk factors for breast cancer (14).

Diet/dietary supplements. Diet was assessed using a food frequency questionnaire designed specifically for the WHI (17). Personal dietary supplement use was assessed by means of an interviewer-administered supplement inventory form (18, 20). Total vitamin D intake was calculated as food plus supplements.

Exposure to solar ultraviolet radiation. Solar irradiation exposure was assessed for each WHI clinical center in langleys (gram-calories per cm^2) and watts (J/second per m^2). Langleys measure the mean amount of solar radiation that reaches the ground annually; this is an estimate of sunlight exposure (10). Watts are used to estimate the daily ultraviolet B flux reaching the earth within the range required for cutaneous synthesis of vitamin D; 1989 data for watts were obtained from the National Aeronautics and Space Administration (10).

Anthropometry. Height, weight, and waist and hip circumferences were measured at baseline and at each visit thereafter. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. For these analyses, the BMI used was that which corresponded to the blood draw date.

Lifestyle habits and demographic characteristics. Participants completed a baseline questionnaire for usual recreational physical activity, walking, smoking, and alcohol intake. Age and race/ethnicity were ascertained by self-report.

Table 2. Continued

Characteristic	Quartile of Serum 25-Hydroxyvitamin D Concentration, nmol/L												*P* Value
	≥64.9			50.9–<64.9			36.7–<50.9			<36.7			
	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	No.	%	Mean (SD)	
Total vitamin D intake, IU/day													
<200	115	23.2		139	27.0		203	35.6		337	60.9		<0.001
200–<400	94	19.0		116	22.6		110	19.3		87	15.7		
400–<600	130	26.2		149	29.0		156	27.3		87	15.7		
≥600	157	31.7		110	21.4		102	17.9		42	7.6		
Alcohol intake													
Nondrinker	54	10.9		46	8.9		58	10.1		64	11.5		<0.001
Past drinker	62	12.5		92	17.8		92	16.0		104	18.8		
<1 drink/month	61	12.3		65	12.6		73	12.7		107	19.3		
<1 drink/week	96	19.4		102	19.7		138	24.0		110	19.9		
1–7 drinks/week	151	30.5		154	29.8		153	26.6		111	20.0		
>7 drinks/week	71	14.3		58	11.2		62	10.8		58	10.5		
Mammogram within past 2 years													
No	142	28.4		146	28.1		186	32.4		194	34.5		0.062
Yes	358	71.6		373	71.9		389	67.7		368	65.5		
Use of hormone therapy at blood draw													
None/past use	172	34.4		229	44.0		263	45.5		277	49.3		<0.001
Current use													
Estrogen alone	129	25.8		120	23.1		154	26.6		123	21.9		
Estrogen + progesterone	199	39.8		171	32.9		161	27.9		162	28.8		

Abbreviations: MET, metabolic equivalent; SD, standard deviation.
[a] Cases and controls were matched on age, clinical center location, race/ethnicity, and date of blood collection.
[b] Weight (kg)/height $(m)^2$.

Statistical analysis

Conditional logistic regression was used to compute odds ratios and 95% confidence intervals for colorectal and breast cancer risk. All models were conditioned on the case-control matching factors (age, latitude of WHI clinical center, race/ethnicity, and date of blood collection). We built a set of models for each cancer that successively added covariates known to influence serum 25(OH)D concentrations, breast cancer risk, or colorectal cancer risk (10, 11). Variables were added in the order of their known or hypothesized strength as potential confounders. The adjusted models included randomization assignment (calcium-vitamin D or placebo), BMI (log-transformed, continuous), leisure physical activity (tertiles of metabolic equivalent (MET)-hours/week), smoking (past, current, or never), alcohol intake, and postmenopausal hormone therapy. For the colorectal cancer models, additional covariates included colorectal cancer screening within the previous 5–6 years (yes/no), family history of colorectal cancer (yes/no), red meat intake, and total calcium intake (food + supplements). The primary exposure, serum 25(OH)D, was modeled as a categorical variable. For the breast cancer models, additional covariates included having undergone a mammogram within the previous 2 years (yes/no) and 5-year Gail risk score (age at menopause, age at first livebirth, number of previous breast biopsies, number of first-degree relatives with breast cancer) (21). Additional variables were examined in relation to both cancer outcomes, but they were not included in the final models because they were either highly collinear with other covariates (season of blood collection, waist:hip ratio) or were neither influential in parameter estimates nor statistically significant (langleys and watts) (10).

We also investigated whether the main effects for associations of serum 25(OH)D with risk of either colorectal cancer or breast cancer were modified by physical activity, BMI, postmenopausal hormone therapy, or use of vitamin D-containing dietary supplements. Multiplicative interaction was tested by creating categories for each potential effect modifier (tertiles of physical activity (MET-hours/week); BMI categories (normal (<25.0), overweight (25.0–29.9), or obese (≥30.0)); never use of hormone therapy vs. ever use; never/past use of hormone therapy vs. current use; baseline use of vitamin D-containing dietary supplements (≥100 IU/day) vs.

nonuse). We constructed linear terms by creating a cross-product of each of these categories with the median values of the quartiles of the serum 25(OH)D distribution. Evidence for effect modification was tested using the Wald chi-square test (1 df). In the BMI-stratified models, BMI was also included as a continuous variable within each stratum to further control for confounding by BMI. All tests were 2-sided, with $P < 0.05$ as the criterion for statistical significance. Analyses were conducted using SAS, version 9.2 (SAS Institute, Inc., Cary, North Carolina).

RESULTS

The associations between various demographic and health-related characteristics and serum concentrations of 25(OH)D provide information about factors that may confound the associations of serum 25(OH)D with breast and colorectal cancer risk (Tables 1 and 2). The 25(OH)D quartile cutpoints for these analyses were based on the distribution among the controls for each case-control group, and since these distributions differed for breast and colorectal cancer groups, results are presented in separate tables. A priori, we considered factors previously shown to be predictors of serum 25(OH)D in WHI (10), as well as factors known to be associated with breast or colorectal cancer risk (14). The case-control matching factors were not included as covariates in the models (see Materials and Methods section) but are presented in these tables as informational.

Across the colorectal cancer case-control group, statistically significant differences in serum 25(OH)D concentration were observed by waist circumference, race/ethnicity, BMI, recreational physical activity (MET-hours/week), total vitamin D intake (food + supplements), total calcium intake (food + supplements), alcohol intake, and use of postmenopausal hormone therapy. In the breast cancer case-control group, the demographic and lifestyle characteristics associated with serum 25(OH)D included age, waist circumference, race/ethnicity, BMI, recreational physical activity, total vitamin D intake, alcohol intake, and postmenopausal hormone therapy.

Table 3 provides results for associations of serum 25(OH)D with colorectal cancer risk. We began with the basic model (case-control matching factors) and successively added the variables thought most likely to confound the associations: trial arm assignment, BMI, and physical activity. Subsequent models included smoking, colorectal cancer screening, family history of colorectal cancer, postmenopausal hormone therapy, alcohol intake, and red meat intake. Little variation in the odds ratio estimates was observed as the initial covariates were added to the model, but the odds ratios strengthened as the final, full model was built. In the fully adjusted model, compared with participants with a high serum 25(OH)D concentration, those with low serum 25(OH)D values had a 4.5-fold increased risk of colorectal cancer (odds ratio (OR) = 4.45, 95% confidence interval (CI): 1.96, 10.10; P-trend = 0.003). We also examined additional measures of adiposity in the model, such as adding waist circumference and replacing BMI with waist circumference (data not shown). However, since there were no meaningful differences in the parameter estimates, we retained only BMI in the final model as shown.

Associations of serum 25(OH)D with invasive breast cancer are presented in Table 4. The model-building followed the same approach as that for colorectal cancer. The odds ratio for invasive breast cancer was 1.33 (95% CI: 1.02, 1.72; P-trend = 0.01) in the basic model, with nearly identical results for the model adjusted only for CaD arm. When BMI and physical activity were added to the model, the results were strongly attenuated. Most of the subsequent odds ratios hovered around the null value of 1.0, and 95% confidence intervals included 1.0. The final model was adjusted for CaD arm, BMI, physical activity, smoking, mammography, Gail 5-year risk score, use of hormone therapy, and alcohol intake. In subgroup analyses restricted to estrogen receptor-positive breast cancer, results did not differ (data not shown).

We next investigated whether associations of serum 25(OH)D with either breast cancer risk or colorectal cancer risk were modified by use of hormone therapy, BMI, physical activity, or use of vitamin D-containing dietary supplements (Table 5). While the colorectal cancer odds ratios for low (vs. high) serum 25(OH)D were stronger for never or past users of hormone therapy than for current users (OR = 4.12 and OR = 2.19, respectively), the P value for the formal interaction test was not statistically significant ($P = 0.56$). The association of 25(OH)D with colorectal cancer risk differed by BMI (P for interaction = 0.01). For women of normal weight (BMI <25.0), the colorectal cancer odds ratios were nonsignificant and relatively constant across the 25(OH)D distribution, whereas for women with BMIs of 25.0–29.9, an increased risk was observed for women in the lowest quartile of serum 25(OH)D (OR = 3.75, 95% CI: 1.25, 11.25) compared with the referent. For obese women (BMI ≥30.0), increased risks were observed across the quartiles, but the greatest risk was seen among obese women in the lowest quartile of serum 25(OH)D concentration (OR = 7.43, 95% CI: 2.10, 26.27). There was no evidence of effect modification by physical activity (P for interaction = 0.85) or use of vitamin D-containing dietary supplements (P for interaction = 0.82). For breast cancer, there was no evidence for effect modification by hormone therapy use, BMI, physical activity, or use of vitamin D-containing dietary supplements.

DISCUSSION

Our objective in this study was to gain insight into the analytic approaches that should be considered when testing associations between serum 25(OH)D and cancer risk in postmenopausal women. In this nested case-control study from the WHI CaD Trial, women with a low serum 25(OH)D concentration, versus those with a high concentration, had over a 4-fold increased risk of colorectal cancer. Our careful and systematic statistical adjustment for health and lifestyle variables thought to confound these associations, such as BMI and physical activity, had very little influence on the odds ratio estimates, and the associations remained strong and robust in all models. Further, the associations were not modified by postmenopausal hormone therapy, physical activity, or use of

Table 3. Association of Serum 25-Hydroxyvitamin D Concentration With Invasive Colorectal Cancer Risk and the Strength of Covariates, Women's Health Initiative, 1994–2005

Model and Variables Included	Quartile[a] of 25-Hydroxyvitamin D Concentration, nmol/L								*P* Trend[b]
	≥64.5 (231 Cases, 269 Controls)		43.6–<64.5 (250 Cases, 270 Controls)		32.7–<43.6 (306 Cases, 272 Controls)		<32.7 (293 Cases, 269 Controls)		
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Unadjusted[c]	1.0	Referent	1.97	1.16, 3.35	1.49	0.88, 2.52	2.72	1.55, 4.77	0.003
WHI intervention arm	1.0	Referent	1.96	1.15, 3.34	1.48	0.87, 2.51	2.72	1.55, 4.77	0.003
WHI intervention arm, BMI	1.0	Referent	1.95	1.14, 3.31	1.48	0.87, 2.47	2.62	1.48, 4.64	0.005
WHI intervention arm, BMI, physical activity	1.0	Referent	2.10	1.16, 3.78	1.42	0.79, 2.55	2.80	1.50, 5.23	0.007
WHI intervention arm, BMI, physical activity, smoking	1.0	Referent	2.06	1.13, 3.75	1.47	0.81, 2.66	3.04	1.61, 5.76	0.003
WHI intervention arm, BMI, physical activity, smoking, CRC screening within the past 5–6 years	1.0	Referent	2.10	1.15, 3.85	1.48	0.82, 2.69	3.08	1.62, 5.86	0.003
WHI intervention arm, BMI, physical activity, smoking, family history of CRC	1.0	Referent	1.97	1.00, 3.90	1.20	0.61, 2.34	2.79	1.37, 5.68	0.02
WHI intervention arm, BMI, physical activity, smoking, HRT use	1.0	Referent	2.11	1.15, 3.85	1.52	0.83, 2.77	3.21	1.67, 6.19	0.003
WHI intervention arm, BMI, physical activity, smoking, CRC screening within the past 5–6 years, family history of CRC, HRT use	1.0	Referent	2.20	1.08, 4.48	1.34	0.67, 2.67	3.26	1.54, 6.89	0.01
WHI intervention arm, BMI, physical activity, smoking, CRC screening within the past 5–6 years, family history of CRC, HRT use, alcohol intake	1.0	Referent	2.51	1.21, 5.21	1.24	0.61, 2.51	3.18	1.49, 6.78	0.02
WHI intervention arm, BMI, physical activity, smoking, CRC screening within the past 5–6 years, family history of CRC, HRT use, red meat intake	1.0	Referent	2.27	1.09, 4.73	1.36	0.67, 2.77	3.67	1.68, 8.00	0.006
WHI intervention arm, BMI, physical activity, smoking, CRC screening within the past 5–6 years, family history of CRC, HRT use, alcohol intake, red meat intake	1.0	Referent	2.39	1.15, 5.00	1.33	0.65, 2.72	3.55	1.62, 7.77	0.009
WHI intervention arm, BMI, physical activity, smoking, CRC screening within the past 5–6 years, family history of CRC, HRT use, alcohol intake, red meat intake, total calcium intake	1.0	Referent	2.76	1.30, 5.89	1.51	0.72, 3.14	4.45	1.96, 10.10	0.003

Abbreviations: BMI, body mass index; CRC, colorectal cancer; HRT, hormone replacement therapy; WHI, Women's Health Initiative.

[a] Quartile cutpoints were based on the distribution of 25-hydroxyvitamin D concentrations in the control group.

[b] Trend test based on a linear term using the median values of the quartiles.

[c] Cases and controls were matched on age, clinical center location, race/ethnicity, and date of blood collection. See Materials and Methods section for details.

vitamin D-containing dietary supplements. However, we did observe modest effect modification of the 25(OH)D-colorectal cancer association by BMI. Conversely, we observed no association of serum 25(OH)D with either total breast cancer risk or estrogen receptor-positive breast cancer.

Our principal goal was to explore the extent to which behavioral and lifestyle factors influenced the associations of serum 25(OH)D with colorectal and breast cancer risk. The odds ratio estimates for colorectal cancer varied little when health and lifestyle covariates were added to the

Table 4. Association of Serum 25-Hydroxyvitamin D Concentration With Invasive Breast Cancer Risk and the Strength of Covariates, Women's Health Initiative, 1994–2005

Model and Variables Included	Quartile[a] of 25-Hydroxyvitamin D Concentration, nmol/L								*P* Trend[b]
	≥64.9 (53 Cases, 77 Controls)		50.9–<64.9 (84 Cases, 78 Controls)		36.7–<50.9 (68 Cases, 79 Controls)		<36.7 (105 Cases, 76 Controls)		
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	
Unadjusted[c]	1.0	Referent	1.09	0.85, 1.41	1.35	1.05, 1.74	1.33	1.02, 1.72	0.01
WHI intervention arm	1.0	Referent	1.09	0.85, 1.41	1.36	1.06, 1.75	1.32	1.02, 1.71	0.01
WHI intervention arm, BMI	1.0	Referent	1.05	0.81, 1.41	1.27	0.99, 1.64	1.19	0.92, 1.56	0.10
WHI intervention arm, BMI, physical activity	1.0	Referent	1.00	0.76, 1.31	1.11	0.84, 1.46	1.05	0.78, 1.40	0.63
WHI intervention arm, BMI, physical activity, smoking	1.0	Referent	0.97	0.74, 1.29	1.11	0.84, 1.48	1.03	0.76, 1.38	0.70
WHI intervention arm, BMI, physical activity, smoking, mammography within the past 2 years	1.0	Referent	0.97	0.73, 1.28	1.10	0.83, 1.46	1.03	0.76, 1.38	0.71
WHI intervention arm, BMI, physical activity, smoking, Gail 5-year risk score	1.0	Referent	0.99	0.75, 1.31	1.13	0.85, 1.50	1.04	0.78, 1.40	0.63
WHI intervention arm, BMI, physical activity, smoking, HRT use	1.0	Referent	0.98	0.74, 1.29	1.12	0.84, 1.49	1.04	0.77, 1.40	0.62
WHI intervention arm, BMI, physical activity, smoking, mammography within the past 2 years, Gail 5-year risk score, HRT use	1.0	Referent	0.99	0.75, 1.32	1.12	0.84, 1.50	1.05	0.78, 1.42	0.58
WHI intervention arm, BMI, physical activity, smoking, mammography within the past 2 years, Gail 5-year risk score, HRT use, alcohol intake	1.0	Referent	0.99	0.75, 1.31	1.11	0.83, 1.49	1.06	0.78, 1.43	0.60

Abbreviations: BMI, body mass index; HRT, hormone replacement therapy; WHI, Women's Health Initiative.

[a] Quartile cutpoints were based on the distribution of 25-hydroxyvitamin D concentrations in the control group.

[b] Trend test based on a linear term using the median values of the quartiles.

[c] Cases and controls were matched on age, clinical center location, race/ethnicity, and date of blood collection. See Materials and Methods section for details.

models. For breast cancer, adjustments attenuated the association. Our initial data exploration revealed that serum concentration of 25(OH)D differed by race/ethnicity, physical activity, BMI, waist circumference, and age, similar to findings in other cohorts (11). However, in a previous analysis, these predictive factors together explained only 21% of the variation in 25(OH)D concentrations in this sample of WHI participants (10), suggesting that much remains to be learned about predictors of serum 25(OH)D. For example, genetic factors probably also contribute to between-person variation in serum 25(OH)D concentrations (12) and perhaps increased risk of colorectal or breast cancer. The lack of strong differences in the odds ratio estimates after addition of the covariates to the models for colorectal cancer suggests that such factors' role as potential confounders of these vitamin D-cancer associations may be less important than previously thought. However, for breast cancer, these factors do appear to confound associations. Clearly, the relations between vitamin D and its predictors and the ensuing relation to cancer outcomes are complex and may differ by cancer site, necessitating careful analytic approaches for various cancers that may be affected differently by certain lifestyle factors. For example, we observed modest effect modification of the serum 25(OH)D-colorectal cancer association by BMI. For obese women in the lowest quartile of serum 25(OH)D, the risk of colorectal cancer was 7 times that for obese women in the highest quartile group. Conversely, we observed no differences in risk estimates across the serum 25(OH)D distributions for women with BMI <25.0.

The data in this report confirm previous WHI findings and are similar to reports from other studies in which lower serum vitamin D and/or lower intake of vitamin D appeared to be associated with increased colorectal cancer risk (2, 22–24). Evidence related to vitamin D and breast cancer risk is less consistent (25). Many previous studies of both cancers have used surrogates of vitamin D exposure, such as diet, dietary supplements, and sunlight exposure, rather than serum measures (3, 23, 26–29). Relatively few prospective studies have examined biomarkers of vitamin D status as we have done here. In the Nurses' Health Study, high (vs. low)

Table 5. Modification of the Relation of Serum 25-Hydroxyvitamin D Concentration With Invasive Colorectal and Breast Cancer Risk by Postmenopausal Hormone Use, Body Mass Index, and Physical Activity, Women's Health Initiative, 1994–2005

Colorectal Cancer[a]	Quartile of 25-Hydroxyvitamin D Concentration, nmol/L							
	≥64.5		43.6–<64.5		32.7–<43.6		<32.7	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Use of hormone replacement therapy								
Never/past use	1.0	Referent	2.83	1.03, 7.79	1.31	0.54, 3.17	4.12	1.60, 10.63
Current use	1.0	Referent	1.60	0.66, 3.88	1.44	0.56, 3.72	2.19	0.83, 5.78
Body mass index[b]*								
<25.0	1.0	Referent	1.21	0.43, 3.46	0.61	0.20, 1.84	1.10	0.33, 3.67
25.0–29.9	1.0	Referent	1.93	0.69, 5.39	1.64	0.53, 5.13	3.75	1.25, 11.25
≥30.0	1.0	Referent	4.72	1.20, 18.63	3.59	0.97, 13.33	7.43	2.10, 26.27
Physical activity, MET-hours/week								
≤3.5	1.0	Referent	1.58	0.44, 5.63	1.75	0.56, 5.52	3.57	1.12, 11.39
>3.5–≤12.5	1.0	Referent	3.11	0.96, 10.05	1.01	0.30, 3.43	2.74	0.85, 8.86
>12.5	1.0	Referent	1.30	0.48, 3.53	1.31	0.48, 3.57	2.33	0.82, 6.63
Use of vitamin D dietary supplements[c]								
Yes	1.0	Referent	2.83	1.03, 7.79	1.31	0.54, 3.17	4.12	1.60, 10.63
No	1.0	Referent	1.60	0.66, 3.88	1.44	0.56, 3.72	2.19	0.83, 5.78

Breast Cancer[d]	Quartile of 25-Hydroxyvitamin D Concentration, nmol/L							
	≥64.9		50.9–<64.9		36.7–<50.9		<36.7	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Use of hormone replacement therapy								
Never/past use	1.0	Referent	1.06	0.68, 1.65	1.25	0.78, 1.99	1.45	0.91, 2.32
Current use								
Estrogen alone	1.0	Referent	1.02	0.57, 1.82	1.41	0.80, 2.48	0.73	0.41, 1.33
Estrogen plus progesterone	1.0	Referent	0.93	0.58, 1.49	0.90	0.56, 1.44	0.92	0.57, 1.50
Body mass index								
<25.0	1.0	Referent	0.83	0.51, 1.35	1.15	0.69, 1.94	0.81	0.44, 1.50
25.0–29.9	1.0	Referent	1.12	0.71, 1.79	0.93	0.59, 1.47	1.02	0.64, 1.61
≥30.0	1.0	Referent	0.99	0.59, 1.67	1.32	0.78, 2.21	1.18	0.71, 1.97
Physical activity, MET-hours/week								
≤3.5	1.0	Referent	0.86	0.51, 1.45	1.04	0.62, 1.74	0.83	0.50, 1.37
>3.5–≤12.5	1.0	Referent	0.92	0.57, 1.50	1.07	0.66, 1.72	1.11	0.67, 1.82
>12.5	1.0	Referent	1.14	0.72, 1.82	1.15	0.71, 1.85	1.31	0.77, 2.23
Use of vitamin D dietary supplements[c]								
Yes	1.0	Referent	1.15	0.75, 1.78	1.23	0.79, 1.90	1.14	0.75, 1.73
No	1.0	Referent	0.89	0.62, 1.27	1.07	0.74, 1.54	0.99	0.62, 1.56

Abbreviations: CI, confidence interval; MET, metabolic equivalent; OR, odds ratio; WHI, Women's Health Initiative.

* P for interaction = 0.01 (Wald χ^2 test (1 df)).

[a] The colorectal cancer analysis included 310 cases and 310 controls. Colorectal cancer models were adjusted for WHI intervention arm, body mass index, smoking, physical activity (except for physical activity model), colorectal cancer screening within the past 5–6 years, family history of colorectal cancer, and use of hormone therapy (except for hormone therapy use models).

[b] Weight (kg)/height $(m)^2$.

[c] Use was defined as ≥100 IU/day.

[d] The breast cancer analysis included 1,080 cases and 1,080 controls. Breast cancer models were adjusted for WHI intervention arm, body mass index, physical activity (except for physical activity models), smoking, mammography within the previous 2 years, Gail 5-year risk score, and use of hormone therapy (except for hormone therapy models).

serum 25(OH)D was associated with a 47% reduced risk of colorectal cancer. The associations were strongest for women older than 60 years and for tumors in the distal colon and the rectum (30). Of 2 earlier studies, a small case-control study

found a strong inverse association of high (vs. low) serum 25(OH)D with colon cancer risk (24), while a nested case-control study including 57 cases found no association (31).

Similar to investigations of colorectal cancer, most studies of vitamin D and breast cancer risk have relied on self-reported information for data on diet, dietary supplements, and sun exposure (32–36), but fewer have used a biomarker of vitamin D status. In a 2006 review of vitamin D, calcium, and breast cancer risk, Cui and Rohan (25) reported on 4 observational studies that examined associations between biomarkers of vitamin D status and breast cancer risk, but only 2 had used serum 25(OH)D as the biomarker of exposure. Of these, 1 found a breast cancer odds ratio of 5.8 (95% CI: 2.3, 14.7) for women with serum 25(OH)D concentrations less than 50 nmol/L compared with those with concentrations of 50 nmol/L or more (37). The other was a nested case-control study which demonstrated a modest inverse association of serum 25(OH)D with breast cancer risk (38). More recently, in a meta-analysis of breast cancer case-control and nested case-control studies (39), the overall summary relative risk was 0.89 for each 10-ng/mL increase in serum 25(OH)D concentration, but the authors concluded that only prospective studies should be used for inferences regarding breast cancer risk (largely because of the timing of the blood draws in the case-control studies and other design limitations). The summary relative risk for the 5 cohort studies was 0.97 (95% CI: 0.92, 1.03) (39). Low serum 25(OH)D concentration was associated with breast cancer-related mortality in one study (40) but not in another (41).

We and other investigators (39) have no firm explanation for why the accumulating evidence suggests that lower serum 25(OH)D concentrations are associated with increased risk of colorectal cancer but not breast cancer. This evidence is strengthened by relatively consistent data demonstrating inverse associations of serum 25(OH)D with precursor conditions for colorectal cancer (i.e., adenomas) (42–44) but not precursors for breast cancer (i.e., breast density) (45, 46). One reason for the differential associations by cancer site may be that vitamin D plays a unique, direct role in the human gut, both to maintain calcium and phosphorus homeostasis and to bind bile acids (47, 48). Whether these functions have an independent role in relation to carcinogenesis that may not exist in other tissues is unknown.

Strengths of this study include the prospective nature of the study and the well-characterized WHI population with extensive details on health, demographic, and lifestyle characteristics, which allowed us to explore the role of several lifestyle factors in the associations. Further, WHI has comprehensive monitoring of cancer screening tests and central adjudication of cancer endpoints. Limitations include a single assessment of serum 25(OH)D concentration. Some evidence suggests that use of more than 1 measure may improve precision (49, 50). However, in a cohort study, Jorde et al. (51) tracked serum 25(OH)D concentrations among 2,668 participants over 14 years and found that serum 25(OH)D remained relatively stable over time. Finally, as in all observational studies, residual confounding may exist, particularly in relation to variables that were measured with error or variables that were not measured at all in WHI.

In conclusion, in 2 case-control studies nested within the WHI CaD Trial, a low (vs. high) serum 25(OH)D concentration was associated with an over 4-fold increased risk of colorectal cancer after multivariable adjustment. However, we observed no association of serum 25(OH)D concentrations with breast cancer risk, similar to a previous finding (16). We sought to determine whether several plausible lifestyle variables confounded the associations; such evidence was found for breast cancer but not for colorectal cancer. The basis for the divergent response to multivariable adjustment remains uncertain, but these results may suggest different biologic pathways for vitamin D across cancer sites. Still, one of the important questions yet to be answered is whether the observed associations with colorectal cancer are causal or merely reflective of other, unmeasured or inadequately measured confounding variables associated with healthy behaviors, such as regular physical activity, good dietary habits, and healthy body weight. Whether interventions designed to change serum 25(OH)D status will reduce risk of either colorectal cancer or breast cancer remains unknown. It is crucial to address these questions in order to offer the most appropriate advice to clinicians and their patients regarding vitamin D's role in cancer prevention.

ACKNOWLEDGMENTS

Author affiliations: Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington (Marian L. Neuhouser, Mary Pettinger); Department of Preventive Medicine, Brigham and Women's Hospital, Harvard School of Public Health, Boston, Massachusetts (JoAnn E. Manson); University at Buffalo, State University of New York, Buffalo, New York (Amy Millen, Jean Wactawski-Wende); Health Partners Research Foundation, Minneapolis, Minnesota (Karen Margolis); Arizona Cancer Center, University of Arizona, Tucson, Arizona (Elizabeth T. Jacobs); Division of Preventive Medicine, School of Medicine, University of Alabama at Birmingham, Birmingham, Alabama (James M. Shikany); Department of Epidemiology and Medicine, School of Medicine, Wake Forest University, Winston-Salem, North Carolina (Mara Vitolins); Lombardi Comprehensive Cancer Center, Georgetown University, Washington, DC (Lucile Adams-Campbell); Program on Genomics and Nutrition, School of Public Health, University of California, Los Angeles, Los Angeles, California (Simin Liu); Center for Health Research, Kaiser Permanente, Portland, Oregon (Erin LeBlanc); and Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, Tennessee (Karen C. Johnson).

The Women's Health Initiative (WHI) is funded by the National Heart, Lung, and Blood Institute through contracts NO1WH22110, 24152, 32100-2, 32105-6, 32108-9, 32111-13, 32115, 32118-32119, 32122, 42107-26, 42129-32, and 44221.

WHI Program Office: National Heart, Lung, and Blood Institute, Bethesda, Maryland—Jacques Rossouw, Shari Ludlam, Joan McGowan, Leslie Ford, Nancy Geller. *WHI*

Clinical Coordinating Center: Fred Hutchinson Cancer Research Center, Seattle, Washington—Ross Prentice, Garnet Anderson, Andrea LaCroix, Charles Kooperberg; Medical Research Labs, Highland Heights, Kentucky—Evan Stein; University of California, San Francisco, San Francisco, California—Steven Cummings. *WHI clinical centers*: Albert Einstein College of Medicine, Bronx, New York—Sylvia Wassertheil-Smoller; Baylor College of Medicine, Houston, Texas—Haleh Sangi-Haghpeykar; Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts—JoAnn E. Manson; Brown University, Providence, Rhode Island—Charles B. Eaton; Emory University, Atlanta, Georgia—Lawrence S. Phillips; Fred Hutchinson Cancer Research Center, Seattle, Washington—Shirley Beresford; George Washington University Medical Center, Washington, DC—Lisa Martin; Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California—Rowan Chlebowski; Kaiser Permanente Center for Health Research, Portland, Oregon—Erin LeBlanc; Kaiser Permanente Division of Research, Oakland, California—Bette Caan; Medical College of Wisconsin, Milwaukee, Wisconsin—Jane Morley Kotchen; MedStar Research Institute/Howard University, Washington, DC—Barbara V. Howard; Northwestern University, Chicago, Illinois—Linda Van Horn; Rush Medical Center, Chicago, Illinois—Henry Black; Stanford Prevention Research Center, Stanford, California—Marcia L. Stefanick; University at Stony Brook, State University of New York, Stony Brook, New York—Dorothy Lane; Ohio State University, Columbus, Ohio—Rebecca Jackson; University of Alabama at Birmingham, Birmingham, Alabama—Cora E. Lewis; University of Arizona, Tucson, Arizona—Cynthia A. Thomson; University at Buffalo, State University of New York, Buffalo, New York—Jean Wactawski-Wende; University of California, Davis, Sacramento, California—John Robbins; University of California, Irvine, Irvine, California—F. Allan Hubbell; University of California, Los Angeles, Los Angeles, California—Lauren Nathan; University of California, San Diego, LaJolla, California—Robert D. Langer; University of Cincinnati, Cincinnati, Ohio—Margery Gass; University of Florida, Gainesville, Florida—Marian Limacher; University of Hawaii, Honolulu, Hawaii—J. David Curb; University of Iowa, Iowa City, Iowa—Robert Wallace; University of Massachusetts/Fallon Clinic, Worcester, Massachusetts—Judith Ockene; University of Medicine and Dentistry of New Jersey, Newark, New Jersey—Norman Lasser; University of Miami, Miami, Florida—Mary Jo O'Sullivan; University of Minnesota, Minneapolis, Minnesota—Karen Margolis; University of Nevada, Reno, Nevada—Robert Brunner; University of North Carolina at Chapel Hill, Chapel Hill, North Carolina—Gerardo Heiss; University of Pittsburgh, Pittsburgh, Pennsylvania—Lewis Kuller; University of Tennessee Health Science Center, Memphis, Tennessee—Karen C. Johnson; University of Texas Health Science Center, San Antonio, Texas—Robert Brzyski; University of Wisconsin, Madison, Wisconsin—Gloria E. Sarto; School of Medicine, Wake Forest University, Winston-Salem, North Carolina—Mara Vitolins; School of Medicine, Wayne State University/Hutzel Hospital, Detroit, Michigan—Michael S. Simon.

Conflict of interest: none declared.

REFERENCES

1. Colston KW, Berger U, Coombes RC. Possible role for vitamin D in controlling breast cancer cell proliferation. *Lancet*. 1989;1(8631):188–191.
2. Lappe JM, Travers-Gustafson D, Davies KM, et al. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. *Am J Clin Nutr.* 2007;85(6):1586–1591.
3. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? *Int J Epidemiol*. 1980;9(3):227–231.
4. Lointier P, Wargovich MJ, Saez S, et al. The role of vitamin D3 in the proliferation of a human colon cancer cell line in vitro. *Anticancer Res*. 1987;7(4B):817–821.
5. Evans SR, Schwartz AM, Shchepotin EI, et al. Growth inhibitory effects of 1,25-dihydroxyvitamin D3 and its synthetic analogue, 1α,25-dihydroxy-16-ene-23yne-26,27-hexafluoro-19-nor-cholecalciferol (Ro 25-6760), on a human colon cancer xenograft. *Clin Cancer Res*. 1998;4(11):2869–2876.
6. Meggouh F, Lointier P, Saez S. Sex steroid and 1,25-dihydroxyvitamin D3 receptors in human colorectal adenocarcinoma and normal mucosa. *Cancer Res*. 1991;51(4):1227–1233.
7. Pirianov G, Colston KW. Interaction of vitamin D analogs with signaling pathways leading to active cell death in breast cancer cells. *Steroids*. 2001;66(3–5):309–318.
8. International Agency for Research on Cancer. *Vitamin D and Cancer.* (IARC Working Group Reports, vol 5). Lyon, France: International Agency for Research on Cancer; 2008.
9. Millen AE, Pettinger M, Freudenheim JL, et al. Incident invasive breast cancer, geographic location of residence, and reported average time spent outside. *Cancer Epidemiol Biomarkers Prev.* 2009;18(2):495–507.
10. Millen AE, Wactawski-Wende J, Pettinger M, et al. Predictors of serum 25-hydroxyvitamin D concentrations among postmenopausal women: the Women's Health Initiative Calcium plus Vitamin D Clinical Trial. *Am J Clin Nutr.* 2010;91(5):1324–1335.
11. McCullough ML, Weinstein SJ, Freedman DM, et al. Correlates of circulating 25-hydroxyvitamin D: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. *Am J Epidemiol*. 2010;172(1):21–35.
12. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet.* 2010;376(9736):180–188.
13. Ross AC, Taylor CL, Yaktine AL, et al, eds. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academies Press; 2011.
14. Women's Health Initiative Study Group. Design of the Women's Health Initiative Clinical Trial and Observational Study. *Control Clin Trials*. 1998;19(1):61–109.
15. Wactawski-Wende J, Kotchen JM, Anderson GL, et al. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N Engl J Med.* 2006;354(7):684–696.
16. Chlebowski RT, Johnson KC, Kooperberg C, et al. Calcium plus vitamin D supplementation and the risk of breast cancer. Women's Health Initiative Investigators. *J Natl Cancer Inst*. 2008;100(22):1581–1591.
17. Patterson RE, Kristal AR, Tinker LF, et al. Measurement characteristics of the Women's Health Initiative food frequency questionnaire. *Ann Epidemiol*. 1999;9(3):178–187.
18. Patterson RE, Levy L, Tinker LF, et al. Evaluation of a simplified vitamin supplement inventory developed for the Women's Health Initiative. *Public Health Nutr.* 1999;2(3):273–276.
19. Curb JD, McTiernan A, Heckbert SR, et al. Outcomes ascertainment and adjudication methods in the Women's Health Initiative. *Ann Epidemiol*. 2003;13(suppl 9):S122–S128.

20. Patterson RE, Kristal AR, Levy L, et al. Validity of methods used to assess vitamin and mineral supplement use. *Am J Epidemiol*. 1998;148(7):643–649.
21. Gail MH, Costantino JP. Validating and improving models for projecting the absolute risk of breast cancer. *J Natl Cancer Inst*. 2001;93(5):334–335.
22. White E, Shannon JS, Patterson RE. Relationship between vitamin and calcium supplement use and colon cancer. *Cancer Epidemiol Biomarkers Prev.* 1997;6(10):769–774.
23. Martínez ME, Giovannucci EL, Colditz GA, et al. Calcium, vitamin D, and the occurrence of colorectal cancer among women. *J Natl Cancer Inst*. 1996;88(19):1375–1382.
24. Garland CF, Comstock GW, Garland FC, et al. Serum 25-hydroxyvitamin D and colon cancer: eight-year prospective study. *Lancet*. 1989;2(8673):1176–1178.
25. Cui Y, Rohan TE. Vitamin D, calcium, and breast cancer risk: a review. *Cancer Epidemiol Biomarkers Prev*. 2006;15(8): 1427–1437.
26. Bostick RM, Potter JD, Sellers TA, et al. Relation of calcium, vitamin D, and dairy food intake to incidence of colon cancer among older women. The Iowa Women's Health Study. *Am J Epidemiol*. 1993;137(12):1302–1317.
27. Kearney J, Giovannucci E, Rimm EB, et al. Calcium, vitamin D, and dairy foods and the occurrence of colon cancer in men. *Am J Epidemiol*. 1996;143(9):907–917.
28. Zheng W, Anderson KE, Kushi LH, et al. A prospective cohort study of intake of calcium, vitamin D, and other micronutrients in relation to incidence of rectal cancer among postmenopausal women. *Cancer Epidemiol Biomarkers Prev.* 1998;7(3):221–225.
29. McCullough ML, Robertson AS, Rodriguez C, et al. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort (United States). *Cancer Causes Control*. 2003;14(1):1–12.
30. Feskanich D, Ma J, Fuchs CS, et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. *Cancer Epidemiol Biomarkers Prev.* 2004;13(9):1502–1508.
31. Braun MM, Helzlsouer KJ, Hollis BW, et al. Colon cancer and serum vitamin D metabolite levels 10–17 years prior to diagnosis. *Am J Epidemiol*. 1995;142(6):608–611.
32. Shin MH, Holmes MD, Hankinson SE, et al. Intake of dairy products, calcium, and vitamin D and risk of breast cancer. *J Natl Cancer Inst*. 2002;94(17):1301–1311.
33. McCullough ML, Rodriguez C, Diver WR, et al. Dairy, calcium, and vitamin D intake and postmenopausal breast cancer risk in the Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol Biomarkers Prev.* 2005;14(12): 2898–2904.
34. Anderson LN, Cotterchio M, Vieth R, et al. Vitamin D and calcium intakes and breast cancer risk in pre- and postmenopausal women. *Am J Clin Nutr.* 2010;91(6): 1699–1707.
35. Robien K, Cutler GJ, Lazovich D. Vitamin D intake and breast cancer risk in postmenopausal women: the Iowa Women's Health Study. *Cancer Causes Control*. 2007;18(7):775–782.
36. Knight JA, Lesosky M, Barnett H, et al. Vitamin D and reduced risk of breast cancer: a population-based case-control study. *Cancer Epidemiol Biomarkers Prev.* 2007;16(3):422–429.
37. Lowe LC, Guy M, Mansi JL, et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. *Eur J Cancer.* 2005;41(8):1164–1169.
38. Bertone-Johnson ER, Chen WY, Holick MF, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2005; 14(8):1991–1997.
39. Gandini S, Boniol M, Haukka J, et al. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. *Int J Cancer.* 2011;128(6):1414–1424.
40. Goodwin PJ, Ennis M, Pritchard KI, et al. Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. *J Clin Oncol*. 2009;27(23):3757–3763.
41. Jacobs ET, Thomson CA, Flatt SW, et al. Vitamin D and breast cancer recurrence in the Women's Healthy Eating and Living (WHEL) Study. *Am J Clin Nutr.* 2011;93(1):108–117.
42. Peters U, Hayes RB, Chatterjee N, et al. Circulating vitamin D metabolites, polymorphism in vitamin D receptor, and colorectal adenoma risk. Prostate, Lung, Colorectal and Ovarian Cancer Screening Project Team. *Cancer Epidemiol Biomarkers Prev.* 2004;13(4):546–552.
43. Platz EA, Hankinson SE, Hollis BW, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and adenomatous polyps of the distal colorectum. *Cancer Epidemiol Biomarkers Prev.* 2000;9(10):1059–1065.
44. Wei MY, Garland CF, Gorham ED, et al. Vitamin D and prevention of colorectal adenoma: a meta-analysis. *Cancer Epidemiol Biomarkers Prev.* 2008;17(11):2958–2969.
45. Knight JA, Vachon CM, Vierkant RA, et al. No association between 25-hydroxyvitamin D and mammographic density. *Cancer Epidemiol Biomarkers Prev.* 2006;15(10):1988–1992.
46. Neuhouser ML, Bernstein L, Hollis BW, et al. Serum vitamin D and breast density in breast cancer survivors. *Cancer Epidemiol Biomarkers Prev.* 2010;19(2):412–417.
47. Holick MF. Vitamin D: the underappreciated D-lightful hormone that is important for skeletal and cellular health. *Curr Opin Endocrinol Diabetes*. 2002;9(1):87–98.
48. Holick MF. Vitamin D. In: Stipanuk MH, ed. *Biochemical and Physiological Aspects of Human Nutrition*. Philadelphia, PA: WB Saunders Company; 2000:624–636.
49. White E, Hunt JR, Casso D. Exposure measurement in cohort studies: the challenges of prospective data collection. *Epidemiol Rev.* 1998;20(1):43–56.
50. Armstrong BK, White E, Saracci R. *Principles of Exposure Measurement in Epidemiology.* Oxford, United Kingdom: Oxford University Press; 1992.
51. Jorde R, Sneve M, Hutchinson M, et al. Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study. *Am J Epidemiol*. 2010;171(8):903–908.