Vitamin D and Breast Cancer Risk: The NHANES I Epidemiologic Follow-up Study, 1971–1975 to 1992[1]

Esther M. John,[2] Gary G. Schwartz, Darlene M. Dreon, and Jocelyn Koo

Northern California Cancer Center, Union City, California 94587 [E. M. J., D. M. D., J. K.], and Sylvester Comprehensive Cancer Center and Department of Epidemiology and Public Health, University of Miami, Miami, Florida 33101 [G. G. S.]

Abstract

We analyzed data from the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study to test the hypothesis that vitamin D from sunlight exposure, diet, and supplements reduces the risk of breast cancer. We identified 190 women with incident breast cancer from a cohort of 5009 white women who completed the dermatological examination and 24-h dietary recall conducted from 1971–1974 and who were followed up to 1992. Using Cox proportional hazards regression, we estimated relative risks (RRs) for breast cancer and 95% confidence intervals, adjusting for age, education, age at menarche, age at menopause, body mass index, alcohol consumption, and physical activity. Several measures of sunlight exposure and dietary vitamin D intake were associated with reduced risk of breast cancer, with RRs ranging from 0.67–0.85. The associations with vitamin D exposures, however, varied by region of residence. The risk reductions were highest for women who lived in United States regions of high solar radiation, with RRs ranging from 0.35–0.75. No reductions in risk were found for women who lived in regions of low solar radiation. Although limited by the relatively small size of the case population, the protective effects of vitamin D observed in this prospective study are consistent for several independent measures of vitamin D. These data support the hypothesis that sunlight and dietary vitamin D reduce the risk of breast cancer.

Introduction

The past 20 years have witnessed a tremendous renaissance in our understanding of the biological roles of vitamin D. Although "classically" vitamin D was considered as a regulator of calcium homeostasis, it is now clear that the hormonal form of vitamin D, $1,25(OH)_2D$,[3] also known as calcitriol, plays far larger roles in the regulation of cellular growth and differentiation (1). Both *in vitro* and *in vivo* studies have demonstrated that $1,25(OH)_2D$ regulates the growth and promotes the differentiation of many types of normal and malignant cells, including human breast cancer cells (reviewed in Ref. 2). The action of $1,25(OH)_2D$ is mediated through its binding to specific intracellular receptors for $1,25(OH)_2D$ (commonly called VDRs) that are members of the steroid/thyroid hormone receptor family (3). VDRs, in turn, bind to DNA sequences called vitamin D response elements, which regulate the transcription of genes involved in cell growth, differentiation, and metastasis (4, 5).

The synthesis of $1,25(OH)_2D$ begins with the cutaneous production of vitamin D after exposure to sunlight or after the intestinal absorption of vitamin D obtained from the diet (6). To become biologically active, vitamin D must undergo two hydroxylation steps: (*a*) at the 25th carbon position, to form 25(OH)D, the major circulating metabolite of vitamin D; and (*b*) at the 1 α position, to form $1,25(OH)_2D$, the hormonal metabolite. Thus, $1,25(OH)_2D$ is unique among steroid hormones in that the initial step in its synthesis is determined by environmental exposures: UV radiation and, to a lesser extent, diet.

There has been considerable recent interest in the potential protective role of vitamin D in the etiology of breast cancer, stimulated, in part, by promising findings in colon (7) and in prostate (8) cancer. Like mortality rates from colon and prostate cancers, mortality rates from breast cancer are higher in the northeastern than in the southern United States (9) and are inversely correlated with solar radiation (9–12). However, regional differences in the prevalence of established risk factors for breast cancer explain only part of the geographic variation in breast cancer mortality rates (13, 14). Epidemiological findings concerning the role of dietary vitamin D and breast cancer risk are inconsistent (15, 16). Furthermore, the interpretation of these data is unclear because, for most individuals, the majority of vitamin D is derived from casual exposure to sunlight (17). To our knowledge, no epidemiological studies have examined the relation between breast cancer and sunlight exposure at the level of the individual.

To examine more fully a possible protective role of vitamin D on breast cancer risk, we analyzed data from a national cohort study, the NHANES I Epidemiologic Follow-up Study. We assessed the relation of sunlight exposure and dietary and supplemental vitamin D intake with subsequent development of breast cancer and report that, among women living in areas of high solar radiation, several measures of sunlight exposure and

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[2] To whom requests for reprints should be addressed, at Northern California Cancer Center, 32960 Alvarado-Niles Road, Suite 600, Union City, CA 94587. Phone: (510) 429-2554; Fax: (510) 429-2550; E-mail: ejohn@nccc.org.

[3] The abbreviations used are: $1,25(OH)_2D$, 1α25-dihydroxyvitamin D; VDR, vitamin D receptor; 25(OH)D, 25-hydroxyvitamin D; NHANES, National Health and Nutrition Examination Survey; USDA, United States Department of Agriculture; RR, relative risk; CI, confidence interval.

dietary vitamin D intake were associated with a 25–65% reduction in breast cancer risk.

Materials and Methods

Study Design

NHANES I was conducted from 1971–1975 in a probability sample of the noninstitutionalized United States population. A wide variety of data were collected through in-person interviews (including sociodemographic background, medical history, 24-h dietary recall, and supplement use), medical examinations (including dermatological examination), and laboratory tests (18, 19).

Adults, ages 25–74 years, including 8596 women, formed the cohort of the NHANES I Epidemiologic Follow-up Study. They were recontacted from 1982–1984 (20), and in 1986 (21), 1987 (22), and 1992 (23) and questioned about various health outcomes, including breast cancer. Of these, 589 women (6.9%) could not be traced or refused to participate in any of the four follow-up surveys, which were conducted in-person (from 1982–1984) or by telephone (in 1986, 1987, and 1992) with surviving individuals or proxy respondents. Medical records were obtained for individuals who reported any hospitalizations during the follow-up period, and death certificates were sought for cohort members who were decreased at follow-up.

Exposure Variables

From the interview, dietary assessment, and dermatological examination we derived the following vitamin D exposure variables:

Usual Sunlight Exposure. Before the baseline dermatological examination, the examining physician inquired about the amount of time spent outdoors at work and during leisure time. Each participant's sunlight exposure was classified as "considerable," "moderate," or "unimpressive."

The 1982–1984 follow-up interview asked participants to rate separately their usual recreational and occupational sunlight exposure as "never," "rare," "occasional," or "frequent." We constructed a measure of overall sunlight exposure (low, medium, high) by classifying women with both frequent occupational and recreational sunlight exposure as "high," women with both rare or no occupational and recreational sunlight exposure as "low," and the women remaining as "medium."

Sun-induced Skin Damage. In the dermatological examination, each participant's actinic skin damage was classified by the physician as "absent," "minimal," "moderate," or "severe." We used this information as a measure of sunlight exposure because, among whites, actinic skin damage is associated with cumulative sunlight exposure (24, 25).

Residential Sunlight Exposure. The baseline interview collected information on region of residence at baseline (*i.e.*, south, west, midwest, and northeast, as defined in Table 2), state of longest residence and duration of residence in that state, and state of birth. Since geographic latitude is an important determinant of cutaneous vitamin D synthesis (26), we estimated average solar radiation levels for each state using data from 235 National Weather Service Stations (27). On the basis of the tertile distribution of average daily total global radiation measured in Langleys, solar radiation in each state was classified as low ($<$305), medium (305–365), or high ($\geq$366).

Dietary Vitamin D Intake. Because the NHANES I nutrient database does not include information on vitamin D, we added vitamin D nutrient values using a methodology developed by Dr. Suzanne Murphy (University of California, Berkeley, CA; Ref. 28). We cross-referenced the foods reported during the 24-h recall interview with the University of California, Berkeley, Minilist nutrient database, which contains nutrients, including vitamin D, for 189 foods (29). We first updated the Minilist with vitamin D values provided in the Provisional Table of vitamin D content, published by the USDA in 1991 (30), and expanded the Minilist with additional foods listed in the USDA Provisional Table that contain vitamin D. For specific fish not included in the USDA Provisional Table and other sources (29, 31, 32), we used substitutions for fish with similar fat content. Fish prepared by different methods (*e.g.*, canned *versus* smoked fish) were assigned identical values. For breakfast cereals, many of which are fortified with vitamin D, we contacted the major manufacturers and obtained information on amount of vitamin D fortification and year when fortification began. Based on the fortification practices in the early 1970s, we assigned vitamin D values to specific brand name cereals reported in the 24-h recall. Because only two types of margarine were fortified with vitamin D in the 1970s, and because the 24-h recall did not record specific types of margarine consumed, we did not assign any vitamin D to margarine. The updated Minilist was then merged with a cross-reference file developed by Dr. Murphy, which assigns vitamin D values to NHANES I single foods using substitutions for NHANES I foods not included in the Minilist and to NHANES I mixed foods using recipes. Using this expanded nutrient database, we estimated each individual's vitamin D intake and, based on the approximate tertile distribution of the analytic cohort, we classified each individual's intake as low ($<$100 IU), medium (100–199 IU), or high ($\geq$200 IU).

Although the baseline interview included a food frequency questionnaire that assessed the usual frequency of consumption (*e.g.*, never, daily, weekly, or less than once a week) during the 3 months preceding the interview, it comprised only 13 food categories (including milk, fish, and eggs), did not assess serving size, and did not distinguish between fish with high or low vitamin D content. We, therefore, limited the dietary analysis to the 24-h recall data.

Supplemental Intake of Vitamin D. The baseline interview inquired about the frequency of supplement use (*i.e.*, daily, weekly but less than daily, or no use) and the type of supplement used (*e.g.*, multivitamins, single vitamin D), although no information on brand name was collected. Since the public use tape-coded only one type of supplement for each supplement user, we obtained a file from the Department of Cancer Prevention at the National Cancer Institute with complete data on all supplements used.

Analytic Cohort

The analytic cohort was established after a series of exclusions. Of the 8007 women, ages 25–74, who were traced and/or participated in at least one of the four follow-up surveys, we excluded 252 women who reported a personal history of cancer and 1586 women without dietary or dermatological data since this information was collected 1971–1974 only. We further excluded 1139 nonwhite women because the number of nonwhite breast cancer cases was too small for separate analysis, and we excluded 21 women with ambiguous information on their personal history of breast cancer. Thus, the analytic cohort included 5009 white women. We identified 191 women with a breast cancer diagnosis during the follow-up period, including 14 cases located through death certificates only. Because not all hospitals participated in the submission of hospital discharge

Table 1 Sunlight exposure and breast cancer risk among white women: NHANES I Epidemiologic Follow-up Study, 1971–1975 to 1992

	Breast cancer cases	Age-adjusted RR (95% CI)	Multivariate-adjusted RR (95% CI)[a]
Sun exposure determined by physician			
Unimpressive	94	1.0	1.0
Moderate	75	0.89 (0.66–1.20)	0.85 (0.63–1.15)
Considerable	20	0.70 (0.43–1.14)	0.70 (0.43–1.14)
P for trend		$P = 0.14$	$P = 0.11$
Actinic skin damage			
None[b]	62	1.0	1.0
None[c]	53	0.95 (0.66–1.37)	0.92 (0.64–1.34)
Minimal	51	0.91 (0.62–1.33)	0.88 (0.60–1.29)
Moderate/severe	24	0.80 (0.49–1.31)	0.80 (0.48–1.29)
P for trend		$P = 0.38$	$P = 0.32$
Recreational sun exposure			
Rare or never	40	1.0	1.0
Occasional	55	0.70 (0.46–1.06)	0.65 (0.43–0.98)
Frequent	60	0.70 (0.47–1.05)	0.66 (0.44–0.99)
P for trend		$P = 0.12$	$P = 0.08$
Occupational sun exposure			
Rare or never	81	1.0	1.0
Occasional	44	1.05 (0.73–1.51)	1.06 (0.73–1.53)
Frequent	29	0.60 (0.39–0.91)	0.64 (0.41–0.98)
P for trend		$P = 0.03$	$P = 0.07$
Combined recreational and occupational sun exposure			
Low	32	1.0	1.0
Medium	99	0.67 (0.45–1.01)	0.81 (0.56–1.17)
High	23	0.50 (0.29–0.86)	0.67 (0.42–1.06)
P for trend		$P = 0.01$	$P = 0.06$

[a] Adjusted for age, education, age at menarche, age at menopause, body mass index, frequency of alcohol consumption, and physical activity.
[b] No actinic skin damage and unimpressive sun exposure, as determined by the physician.
[c] No actinic skin damage and moderate or considerable sun exposure, as determined by the physician.

data, we included all self-reported breast cancer cases in the analysis, given the high reliability of self-reported breast cancer (33, 34).

For the dietary analyses, we excluded individuals who were pregnant or breast-feeding at baseline ($n = 34$) or pregnant during the 3 months preceding the baseline interview ($n = 39$), as well as individuals whose dietary data were provided by a proxy respondent ($n = 141$) or were considered unsatisfactory by the interviewer ($n = 48$). The dietary analyses, therefore, were based on 4747 white women, including 179 breast cancer cases.

Statistical Analysis

Cox proportional hazards regression analyses were performed to assess the relation between exposure to vitamin D from sunlight, diet, and dietary supplements and subsequent development of breast cancer (35). The Statistical Analysis Software procedure PHREG was used to estimate RRs and 95% CIs and to perform tests for trend for each exposure variable. For women with breast cancer, we estimated the person-years of follow-up from the date of the NHANES I interview to the incidence date of breast cancer, defined as the date of first hospital admission related to breast cancer for self-reports confirmed by hospital records, the midpoint of the self-reported year of diagnosis (June 30) for self-reports without hospital record confirmation, and the date of death for breast cancers confirmed by death certificates only. For women without breast cancer, the person-years of follow-up were estimated from the date of the NHANES I interview to the date of last interview, if alive, or to the date of death, if deceased. Average follow-up for the analytic cohort was 17.3 years.

Potential confounding was evaluated for the following risk factors: age, education (<12 years, 12, ≥13), income (quartiles of poverty index), age at menarche (<12 years, 13–14 years, ≥15 years), age at menopause [premenopausal, <45 years, ≥45, based on the classification by Heck and Pamuk (36)], nulliparity/age at first birth (nulliparous, <20 years, 20–24, 25–29, ≥30), body mass index (quartiles of measured weight in kilograms divided by measured height in meters squared), combined measure of occupational and recreational physical activity (low, medium, high), frequency of alcohol consumption during the year preceding the baseline interview (less than once a month or never, once a month to several times a week, almost daily or daily), and family history of breast cancer (yes, no). For the dietary analyses, we also evaluated confounding by calcium intake (<300 IU, 300–599, 600–999, ≥1000) estimated from the 24-h dietary recall. Age at first birth and age at menopause were treated as age-dependent variables.

Individual adjustment for each of these variables (in addition to age) produced no evidence of confounding. To assess the possibility of joint confounding, we performed two sets of multivariate analyses for the subgroup of women with information on nulliparity/age at first birth and family history of breast cancer available from one of the follow-up surveys (142 cases and 3689 noncases). The first set of multivariate analysis controlled for the effect for age, education, income, age at menarche, age at menopause, nulliparity/age at first birth, body mass index, physical activity, alcohol consumption, and family history. The second set adjusted for the same variables except nulliparity/age at first birth, family history, and income. Because the multivariate RRs in the two sets of analyses were essentially the same (data not shown), we performed multivariate analyses for the entire analytic cohort, adjusting for age, education, age at menarche, age at menopause, body mass

Table 2 Residential sun exposure and breast cancer risk among white women: NHANES I Epidemiologic Follow-up Study, 1971–1975 to 1992

	Breast cancer cases	Age-adjusted RR (95% CI)	Multivariate-adjusted RR (95% CI)[a]
Region of residence[b]			
Northeast	51	1.0	1.0
Midwest	48	0.82 (0.55–1.22)	0.83 (0.56–1.23)
West	51	0.81 (0.55–1.20)	0.78 (0.53–1.16)
South	40	0.66 (0.44–1.00)	0.71 (0.47–1.09)
P for trend		*P* = 0.06	*P* = 0.11
Solar radiation at longest residence[c]			
Low	83	1.0	1.0
Medium	67	1.13 (0.82–1.56)	1.17 (0.85–1.62)
High	38	0.70 (0.48–1.03)	0.73 (0.50–1.08)
P for trend		*P* = 0.13	*P* = 0.19
Solar radiation at place of birth			
Low	79	1.0	1.0
Medium	64	0.97 (0.69–1.34)	0.99 (0.72–1.39)
High	35	0.69 (0.46–1.02)	0.73 (0.49–1.09)
P for trend		*P* = 0.08	*P* = 0.16

[a] Adjusted for age, education, age at menarche, age at menopause, body mass index, frequency of alcohol consumption, and physical activity.
[b] Northeast: Maine, Vermont, New Hampshire, Massachusetts, Connecticut, Rhode Island, New York, New Jersey, and Pennsylvania; Midwest: Ohio, Illinois, Indiana, Michigan, Wisconsin, Minnesota, Iowa, and Missouri; West: Washington, Oregon, California, Nevada, New Mexico, Arizona, Texas, Oklahoma, Kansas, Nebraska, North Dakota, South Dakota, Idaho, Utah, Colorado, Montana, and Wyoming; South: Delaware, Maryland, District of Columbia, West Virginia, Virginia, Kentucky, Tennessee, North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, and Arkansas.
[c] Low: Maine, Vermont, New Hampshire, Massachusetts, Connecticut, Rhode Island, New York, Pennsylvania, Ohio, Michigan, Minnesota, and Washington; Medium: New Jersey, Delaware, Maryland, District of Columbia, West Virginia, Virginia, North Carolina, South Carolina, Indiana, Kentucky, Tennessee, Wisconsin, Illinois, Iowa, Missouri, North Dakota, South Dakota, Montana, Oregon; High: Georgia, Florida, Alabama, Mississippi, Arkansas, Louisiana, Nebraska, Kansas, Oklahoma, Texas, Wyoming, Colorado, New Mexico, Idaho, Utah, Arizona, Nevada, and California.

index, physical activity, and alcohol consumption (Tables 1–5). Adjusting the analyses of sunlight exposure for the effect of dietary vitamin D intake and adjusting the analyses of dietary vitamin D intake for the effect of physician-reported sunlight exposure did not alter the results.

Results

Sunlight Exposure. Several measures of sun exposure were associated with reduced breast cancer risk (Table 1). Risk reductions ranged from 20–33% for women with considerable sunlight exposure assessed by physician report (RR = 0.70), moderate to severe sun-induced skin damage (RR = 0.80), and frequent recreational and occupational sunlight exposure assessed by self-report (RR = 0.67). For all three exposure variables, the risk of breast cancer decreased with increasing sunlight exposure.

Similarly, residential solar radiation was inversely associated with breast cancer risk (Table 2). Reduced risks were found for women who lived in the south at baseline (RR = 0.71), whose longest residence was in a state of high solar radiation (RR = 0.73), or were born in a state of high solar radiation (RR = 0.73). Similar risk reductions were found when we restricted the analysis to women who lived 20 or more years (RR = 0.73; 95% CI, 0.49–1.08) or more than half their life-times (RR = 0.69; 95% CI, 0.45–1.04) in a state of high solar radiation.

To distinguish between the effects of sunlight exposure and place of residence, we stratified the analysis by solar radiation level in the state of longest residence. We found that physician-assessed sun exposure and actinic skin damage were not associated with breast cancer risk in areas of low solar radiation, whereas in regions of high solar radiation, the RRs were 0.58 and 0.69, respectively (Table 3). Self-reported recreational and occupational sun exposure, however, did not follow this pattern, with risk reductions observed in both regions of low and medium solar radiation.

Dietary Vitamin D Intake. The average intake of vitamin D from food was slightly lower among breast cancer cases (143 IU) than noncases (148 IU). The difference, however, was not statistically significant. Similarly, the proportion of women with an intake of at least 200 IU was lower among cases (22%) than among noncases (26%). Intake of at least 200 IU from food and daily use of multivitamins were associated with RRs of 0.85 and 0.89, respectively (Table 4). The RR remained unchanged when we restricted the high-exposure category to women with an intake of at least 200 IU or daily use of multivitamins (RR = 0.86).

As noted for the sun exposure variables, dietary vitamin D intake of 200 IU or more decreased breast cancer risk only slightly in regions of low or medium solar radiation (Table 3). For women who lived in regions of high solar radiation, the RR was 0.75.

Sun Exposure and Dietary Intake. When we estimated the RR associated with a combined vitamin D exposure measure (moderate to considerable sun exposure as assessed by physician report and a dietary vitamin D intake of at least 200 IU), we found a slightly greater risk reduction (RR = 0.71; Table 5) than for each of these measures individually (RR = 0.81 and RR = 0.85, respectively). Further limiting the analysis to women who lived in a region of high solar radiation, an even greater reduction in risk was associated with this combined vitamin D exposure measure (RR = 0.36; Table 3).

Discussion

This study sought to test the hypothesis that women with greater exposure to vitamin D, either from sunlight and/or from diet or dietary supplements, would experience a decreased risk of breast cancer. Previous studies of the role of vitamin D in breast cancer have either been ecological studies (9–12) or have considered solely dietary intake (15, 16). We examined the association between breast cancer risk and several measures of vitamin D exposures, including personal sunlight exposure, residential solar radiation, sun-induced skin damage, and dietary intake of vitamin D. To our knowledge, ours is the first study to assess the association between breast cancer and sunlight exposure at the level of the individual. In this cohort analysis, we found that high exposure to sunlight was associated with a 25–65% reduction in breast cancer risk among women whose longest residence was in a state of high solar radiation.

The present investigation has several methodological strengths: the cohort includes a representative sample of the United States population; follow-up was rigorous, and the success of tracing was high (93%); the completion rate of the follow-up interviews was also high, ranging from 91–96% in the four follow-up surveys (20–23); and the baseline data on pertinent risk factors were collected prospectively—considerably in advance of the publication of recent scientific studies implicating a protective role for vitamin D in cancers of the breast (9), colon (7), and prostate (8). Thus, it is very unlikely

Table 3 Sunlight exposure and dietary vitamin D and breast cancer risk among white women, by region of residence: NHANES I Epidemiologic Follow-up Study, 1971–1975 to 1992

	Low solar radiation[a]		Medium solar radiation[a]		High solar radiation[a]	
	Breast cancer cases	Multivariate-adjusted RR (95% CI)[b]	Breast cancer cases	Multivariate adjusted RR (95% CI)[b]	Breast cancer cases	Multivariate-adjusted RR (95% CI)[b]
Sun exposure determined by physician						
Unimpressive	38	1.0	37	1.0	18	1.0
Moderate/considerable	45	1.20 (0.77–1.86)	30	0.71 (0.44–1.15)	19	0.58 (0.30–0.11)
Actinic skin damage						
None	49	1.0	41	1.0	23	1.0
Minimal	23	1.07 (0.64–1.78)	19	0.98 (0.54–1.77)	9	0.77 (0.34–1.73)
Moderate/severe	11	1.18 (0.59–2.36)	7	0.77 (0.33–1.79)	6	0.69 (0.27–1.78)
Dietary vitamin D (IU)						
<100	36	1.0	31	1.0	17	1.0
100–199	18	0.97 (0.55–1.71)	21	1.15 (0.66–2.01)	12	1.20 (0.57–2.53)
≥200	20	0.92 (0.53–1.59)	13	0.91 (0.47–1.75)	7	0.75 (0.31–1.84)
Combined recreational and occupational sun exposure						
Low	15	1.0	9	1.0	8	1.0
Medium	44	0.53 (0.29–0.97)	34	0.83 (0.39–1.76)	19	0.54 (0.23–1.25)
High	9	0.40 (0.17–0.94)	10	0.77 (0.31–1.93)	4	0.35 (0.10–1.20)
MD sun exposure and dietary vitamin D[c]						
Low sun and <200 IU	25	1.0	31	1.0	14	1.0
High sun and ≥200 IU	10	1.13 (0.53–2.43)	9	0.84 (0.40–1.77)	3	0.36 (0.10–1.31)

[a] Level of solar radiation in state of longest residence.
[b] Adjusted for age, education, age at menarche, age at menopause, body mass index, frequency of alcohol consumption, and physical activity.

that knowledge of the research hypothesis under investigation could have biased either the interviewers or study participants.

A large proportion of women in the NHANES I analytic cohort had low vitamin D exposures: 47% of women had a dietary intake of <100 IU, and only 28% of women, ages 24–50, exceeded the current recommended dietary intake of 200 IU (37), which in the United States is the amount present in two cups of "fortified" milk. The low average dietary intake of 148 IU is similar to that of the NHANES III white female population (173 IU),[4] both of which were assessed by a single 24-h dietary recall. Only 18% reported daily use of multivitamins, and 14% reported no or rare occupational and recreational sunlight exposure. These data parallel other studies of the high prevalence of vitamin D insufficiency and deficiency in the United States and elsewhere (38).

Our analysis was based on several measures of sun exposure, the major determinant of vitamin D status. The correlation between these measures was high for the residential sun exposure measures, with correlation coefficients ranging from $r = 0.54$ (for the correlation between region of residence at baseline and solar radiation level in state of birth) to 0.84 (for the correlation between solar radiation level in state of longest residence and solar radiation level in state of birth). These high correlations suggest that within broad regions of residence the study population was geographically relatively stable. For the remaining sun exposure measures, the correlation coefficients were considerably lower, ranging from 0.05 (for the correlation between self-reported sun exposure and actinic skin damage) to 0.24 (for the correlation between physician-assessed sun exposure and actinic skin damage), thus, suggesting independent effects of each exposure measure.

We used self- and physician-reports of personal sunlight exposure as a surrogate for vitamin D status. Although we had no quantitative information on intensity and duration of sunlight exposure or on other factors that influence vitamin D synthesis (*e.g.*, skin pigmentation, use of sun screen and protective clothing, medical conditions, and medications; Ref. 17), self-reported sunlight exposure is a good surrogate for serum levels of 25(OH)D (39–42), the major circulating metabolite of vitamin D. These data support the validity of our exposure measure in ranking individuals according to sunlight exposure. We also used physician-diagnosed actinic skin damage as a measure of personal sunlight exposure. This measure is more objective than self-reported sunlight history and is not dependent on recall.

Finally, we used residential solar radiation as a surrogate measure of vitamin D. The validity of this approach is supported by recent data from NHANES III, conducted from 1988–1994. Serum levels of 25(OH)D were 13% higher in women from the southern United States than from the northern United States (43). These data demonstrate that sunlight exposure variables measured by geographic proxies such as state of residence generally are, indeed, reflective of vitamin D status. Comparisons of serum levels of 25(OH)D among populations in Europe (44), the Netherlands, and Curaçao (45) also found an inverse correlation with geographic latitude, although serum 25(OH)D levels are strongly influenced by local practices (*e.g.*, high intake of oily fish in Scandinavia, clothing habits in Southern Europe, food fortification practices, supplement use; Refs. 46 and 47).

The assessment of the effect of dietary vitamin D intake was limited by several factors. Although 24-h recall is a valid method to assess average nutrient intake in groups of individuals (48) and has been correlated with serum levels of 25(OH)D (38), the estimated nutrient intake during a single day may not represent an individual's usual dietary intake. Similarly, our assessment of the effect of supplemental vitamin D was limited by the lack of information on brand name, which might have introduced exposure misclassification because not all multivitamin preparations contain vitamin D.

The accuracy of an individual's estimated nutrient intake

[4] E. M. John, unpublished data.

Table 4 Dietary and supplemental intake of vitamin D and breast cancer risk among white women: NHANES I Epidemiologic Follow-up Study, 1971–1975 to 1992

	Breast cancer cases	Age-adjusted RR (95% CI)	Multivariate-adjusted RR (95% CI)[a]
Dietary vitamin D			
<100 IU	86	1.0	1.0
100–199 IU	51	1.03 (0.73–1.45)	1.05 (0.74–1.49)
≥200 IU	40	0.84 (0.58–1.23)	0.85 (0.59–1.24)
P for trend		*P* = 0.43	*P* = 0.48
Supplement use (multivitamins or single vitamin D)			
Never	133	1.0	1.0
Weekly	13	0.94 (0.53–1.65)	0.89 (0.50–1.58)
Daily	31	0.93 (0.63–1.38)	0.89 (0.60–1.32)
P for trend		*P* = 0.69	*P* = 0.52
Vitamin D from food or supplements			
<100 IU without daily supplements	73	1.0	1.0
100–199 IU without daily supplements	41	0.98 (0.67–1.44)	1.01 (0.69–1.49)
≥200 IU or daily supplements	63	0.87 (0.62–1.21)	0.86 (0.61–1.20)
P for trend		*P* = 0.40	*P* = 0.37

[a] Adjusted for age, education, age at menarche, age at menopause, body mass index, frequency of alcohol consumption, physical activity, and calcium intake.

Table 5 Vitamin D from sunlight exposure and diet and breast cancer risk among white women: NHANES I Epidemiologic Follow-up Study, 1971–1975 to 1992

	Breast cancer cases	Age-adjusted RR (95% CI)	Multivariate-adjusted RR (95% CI)[a]
Sun exposure and dietary vitamin D[b]			
Low sun and <200 IU	71	1.0	1.0
Low sun and ≥200 IU	18	0.79 (0.57–1.11)	0.75 (0.54–1.06)
High sun and <200 IU	65	0.78 (0.46–1.31)	0.77 (0.46–1.29)
High sun and ≥200 IU	22	0.72 (0.45–1.17)	0.71 (0.44–1.14)
P for trend		*P* = 0.11	*P* = 0.08

[a] Adjusted for age, education, age at menarche, age at menopause, body mass index, frequency of alcohol consumption, physical activity, and calcium intake.
[b] Low sun, unimpressive sun exposure, as determined by the physician; high sun, moderate or considerable sun exposure, as determined by the physician.

also depends on the completeness of the nutrient database. There are no comprehensive nutrient databases that include vitamin D values for all dietary sources, including unsupplemented (*i.e.*, fish liver oil, fatty fish, egg yolk, liver) and supplemented (*i.e.*, milk and certain brand names of breakfast cereal, bread, and margarine) foods (29–32). Furthermore, published nutrient values for fish vary, and the amount of vitamin D in fish depends on geographic location (*e.g.*, Pacific *versus* Atlantic fish) and season (31). The validity of reported vitamin D content in foods fortified with vitamin D is also subject to inaccuracy. Recent studies on the vitamin D content of "fortified" milk in the United States indicated that a significant proportion of the milk analyzed did not contain the 400 IU stated on the label and many samples contained no detectable vitamin D at all (49, 50).

An individual's serum level of 25(OH)D represents short-term vitamin D exposures during the previous few weeks or months (51). A single measurement, therefore, may not reflect habitual long-term sunlight exposure and/or dietary vitamin D intake. The assessment of vitamin D exposures based on self-report or physical examination, as applied in our analysis, may be a better measure of habitual exposure than a single serum measurement. However, the above-discussed sources of error in estimating vitamin D exposures from sunlight and diet may have introduced misclassification of exposure status, although it is highly unlikely that such misclassification would be differential by disease status. The RR estimates would, therefore, tend to be biased toward the null and, thus, would underestimate the actual RRs.

It is possible that our findings may be confounded by other risk factors. Although we cannot exclude the possibility of confounding by unknown risk factors, adjustment for known and suspected risk factors produced very little change in the RRs.

The relatively small number of breast cancer cases (n = 190) limited our ability to detect statistically significant trends of decreasing risk with increasing vitamin D exposure. The findings, however, are consistent for several independent measures of sunlight exposure, which suggests that our exposure variables were measuring the same underlying exposure (*i.e.*, sunlight exposure). The risk reductions we observed were higher for the sunlight exposure variables (20–33%) than for the dietary variables (11–15%). This is as we anticipated because, despite the supplementation of foods, the majority of vitamin D is derived from casual exposure to sunlight. For most persons, casual sunlight exposure provides 80–90% of the body's circulating stores of vitamin D (52). Similarly, the relatively low dietary vitamin D intake in the NHANES I cohort limited our ability to assess the association with much higher dietary intake (*i.e.*, >400 IU). Nevertheless, our findings support the hypothesis that vitamin D may protect against the development of breast cancer.

Our findings are consistent with a diverse literature on the protective effects of vitamin D on breast cancer. The observed lower risk of breast cancer among women living in the south or regions of high solar radiation agrees with several ecological studies (9–12). Our finding of reduced risk among women with a high dietary vitamin D intake also agrees with the results from a recent hospital-based case-control study that reported a significantly lower intake of dietary vitamin D among breast cancer cases than controls (16), although another study found no association (15). A higher dietary vitamin D intake also has been reported among breast cancer patients with euploid (histologically normal) tumors than those with aneuploid (histologically aberrant) tumors (53). None of the dietary studies, however, assessed sunlight exposure, which may have attenuated the association with vitamin D from diet.

Although limited by small case numbers, our findings suggest that the effect of sun exposure and dietary vitamin D intake on breast cancer risk depends on region of residence. No associations were generally found among women who lived in regions of low solar radiation. The risk reductions were highest in regions of high solar radiation and intermediate in regions of medium solar radiation. This finding agrees with studies that have shown that in the northern regions of the United States there is no cutaneous synthesis of vitamin D during the winter months (26).

Because $1,25(OH)_2D$ is the biologically most active metabolite of vitamin D, one might expect breast cancer cases to have lower serum levels of $1,25(OH)_2D$ than noncases. Although one study reported lower concentrations of $1,25(OH)_2D$ in serum among breast cancer patients than controls (54), a recent cohort study reported no association with serum concentrations of $1,25(OH)_2D$ (55). However, in a cross-sectional

study of breast cancer patients, Mawer *et al*. (56) reported a significant decline in serum levels of $1,25(OH)_2D$ with the progression of breast cancer.

This point illustrates a conceptual difficulty that confronts any postulated protective effect of sunlight exposure and/or dietary vitamin D and cancer: although serum levels of 25(OH)D are well correlated with sunlight exposure and dietary vitamin D intake, serum levels of the biologically active hormone $1,25(OH)_2D$ are not. That is, because the hepatic conversion of vitamin D to 25(OH)D is not tightly regulated, individuals with high sunlight exposure have higher circulating levels of 25(OH)D in serum. In contrast, the renal conversion of 25(OH)D to $1,25(OH)_2D$ is tightly regulated. Consequently, in normal individuals, high serum levels of 25(OH)D do not result in correspondingly high serum levels of $1,25(OH)_2D$ (57). Thus, a mechanism(s) by which higher levels of exposure to vitamin D could result in higher levels of $1,25(OH)_2D$ at the level of the breast cell is unclear.

There are several possible explanations to this problem. First, just as geography is a proxy measure for vitamin D metabolite levels in serum, serum levels are proxy measures for vitamin D metabolite levels in tissues. At present, levels of vitamin D metabolites in breast tissue are unknown. However, because many organs sequester steroid hormones, hormone levels in tissue may be much higher than hormone levels in serum (58).

Second, 25(OH)D may not be inactive biologically. Although 25(OH)D binds the VDR with 1/500th–1/1000th the affinity of $1,25(OH)_2D$, it is present in serum at 1000 times the concentration of $1,25(OH)_2D$. Thus, 25(OH)D may exert some $1,25(OH)_2D$-like activity (59).

Finally, it is now clear that organs other than the kidney (*e.g.*, keratinocytes and activated macrophages) possess 1α-hydroxylase activity and can synthesize $1,25(OH)_2D$ locally from 25(OH)D. Recently, Schwartz *et al*. (60) have shown that primary cultures of normal prostate cells, as well as cells from some established prostate cancer cell lines, synthesize $1,25(OH)_2D$ from 25(OH)D. This finding may provide a mechanism for the observed inverse correlation between solar radiation and prostate cancer mortality (61). Caco-2 cells, a human colon adenocarcinoma cell line, also has been shown to possess 1α-hydroxylase activity (62). On the basis of the many similarities between breast and prostate cancer, we speculate that breast cells also possess 1α-hydroxylase activity. If this speculation is correct, extra-renal synthesis of $1,25(OH)_2D$ by breast cells could provide a direct mechanism by which sunlight exposure could decrease the risk of breast cancer, as observed in this analysis. Because the gene encoding 1α-hydroxylase has recently been cloned (*e.g.*, Ref. 63), the question of 1α-hydroxylase activity in breast cells is now amenable to experimental test.

Our findings clearly warrant future epidemiological studies of the etiological role of vitamin D in the development and progression of breast cancer. Such studies would benefit from the use of improved exposure assessments to minimize exposure misclassification, larger case populations to rule out the possibility of chance findings, and ethnically more diverse populations. In addition, such studies should address the important issues of how much sunlight exposure and/or dietary vitamin D might be beneficial and when during life vitamin D exposures might have the greatest effect on breast cancer risk.

In conclusion, the findings of this cohort analysis indicate a protective role of sunlight exposure and dietary vitamin D intake on breast cancer risk among white women. If confirmed, our results would be particularly promising for the primary prevention of breast cancer because dietary vitamin D and casual sunlight exposure are modifiable lifestyle factors. Vitamin D metabolites also have promise as therapeutic agents because $1,25(OH)_2D$ has been shown to inhibit the proliferation and metastasis of both breast (64) and prostate (65–67) cancer cells.

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