



## Original Contribution

# Circulating 25-Hydroxyvitamin D and the Risk of Rarer Cancers: Design and Methods of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers

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The Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (VDPP), a consortium of 10 prospective cohort studies from the United States, Finland, and China, was formed to examine the associations between circulating 25-hydroxyvitamin D (25(OH)D) concentrations and the risk of rarer cancers. Cases (total  $n = 5,491$ ) included incident primary endometrial ( $n = 830$ ), kidney ( $n = 775$ ), ovarian ( $n = 516$ ), pancreatic ( $n = 952$ ), and upper gastrointestinal tract ( $n = 1,065$ ) cancers and non-Hodgkin lymphoma ( $n = 1,353$ ) diagnosed in the participating cohorts. At least 1 control was matched to each case on age, date of blood collection (1974–2006), sex, and race/ethnicity ( $n = 6,714$ ). Covariate data were obtained from each cohort in a standardized manner. The majority of the serum or plasma samples were assayed in a central laboratory using a direct, competitive chemiluminescence immunoassay on the DiaSorin LIAISON platform (DiaSorin, Inc., Stillwater, Minnesota). Masked quality control samples included serum standards from the US National Institute of Standards and Technology. Conditional logistic regression analyses were conducted using clinically defined cutpoints, with 50–<75 nmol/L as the reference category. Meta-analyses were also conducted using inverse-variance weights in random-effects models. This consortium approach permits estimation of the association between 25(OH)D and several rarer cancers with high accuracy and precision across a wide range of 25(OH)D concentrations.

case-control studies; cohort studies; methods; neoplasms; prospective studies; vitamin D

Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CPS-II, Cancer Prevention Study II Nutrition Cohort; IARC, International Agency for Research on Cancer; NCI, National Cancer Institute; NIST, National Institute of Standards and Technology; NYU-WHS, New York University Women's Health Study; 25(OH)D, 25-hydroxyvitamin D; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; VDPP, Cohort Consortium Vitamin D Pooling Project of Rarer Cancers.

Vitamin D has a central role in bone health, and deficient levels are associated with an increased risk of fractures and musculoskeletal symptoms (1–3). Its possible role in other disease outcomes such as cancer is less certain. Most studies of cancer risk and vitamin D status have focused on cancers

at the more common sites, such as colorectal, breast, and prostate cancer, but with the possible exception of colorectal cancer (4), the evidence of an association is inconsistent (4–7). Few studies of less common cancers have been conducted, but of those that have, including investigations of

pancreatic and esophageal cancer, researchers have found both inverse and elevated risks associated with vitamin D (8–10). The possible broad role of vitamin D in overall health, coupled with the high prevalence of vitamin D insufficiency and deficiency in the general population (11, 12), has led to calls for increased vitamin D supplementation and controversial recommendations to increase sun exposure in order to raise vitamin D levels (13). However, because of the potential for harm noted in at least 1 study of a rare but highly lethal cancer (9), additional information concerning the association of vitamin D with rarer cancers is needed.

Clues to the potential role of vitamin D in the development of rarer cancers have come primarily from ecologic studies which observed lower rates of ovarian cancer (11, 14–17), endometrial cancer (18), and non-Hodgkin lymphoma (5), among other cancers, in areas with high sunlight exposure (the main source of vitamin D) as compared with regions with lower sunlight exposure. However, ecologic studies have well-known limitations, including inadequate control for confounding variables and a lack of consideration of other sources of vitamin D exposure. Case-control studies also present challenges, such as the need for rapid case ascertainment, especially for highly lethal cancers, and the identification of a sufficient number of cases to adequately address the proposed research question. Another challenge that cannot be overcome using the traditional retrospective case-control design is the impact of the disease on the exposure, especially one such as vitamin D, where both dietary intake and sun exposure may change as a result of the illness.

Prospective etiologic studies can overcome the limitations of both ecologic and case-control studies through the measurement of vitamin D biomarkers (the best of which is 25-hydroxyvitamin D (25(OH)D) (19)) in blood samples collected years before cancer diagnosis. However, few cohorts contain adequate numbers of any particular rarer cancer, and the collection of serum vitamin D data in a single cohort at a limited geographic location may result in a lack of variability in vitamin D exposure. Collecting and analyzing data from multiple cohort studies conducted at a wide range of latitudes would increase both the number of cases and the variation in 25(OH)D concentrations, improving the ability to elucidate the true association between vitamin D and the development of a rarer cancer.

In May 2007, a conference sponsored by the US National Institutes of Health entitled “Vitamin D and Cancer: Current Dilemmas/Future Needs” was held to critically evaluate the scientific evidence related to vitamin D and cancer risk, identify gaps in knowledge, and determine the type of research needed to make science-based recommendations regarding vitamin D intake/exposure for cancer prevention. In response to a conference recommendation to conduct prospective studies using stored biologic samples, a consortium of prospective cohort studies was formed—the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (VDPP)—to study the association between circulating 25(OH)D concentrations and the development of the following rarer cancers: endometrial, esophageal/gastric, kidney, lymphoma, ovarian, and pancreatic. In this paper, we describe the design and methods of the VDPP.

## MATERIALS AND METHODS

### Overview of the National Cancer Institute VDPP

*National Cancer Institute Cohort Consortium and cohorts participating in the VDPP.* The Cohort Consortium was formed in 2000 under the direction of the National Cancer Institute (NCI) to address the need for large-scale collaborations for the study of genomic associations with cancer (20, 21). The current study is the first project of the Cohort Consortium to examine a serologic factor and address multiple rarer cancer sites. The VDPP includes cohort studies based in China, Finland, and the United States, including 1 with a study center in Hawaii. A common standard nested case-control design was followed, and the vast majority of blood samples for this study (collected between 1974 and 2006) were assayed at a single laboratory, permitting rigorous investigation of the vitamin D hypothesis. The cohorts included in the VDPP, their characteristics, and their contributions to the VDPP data set are described in Table 1 and Table 2. By choice, not all of the participating cohort investigators contributed data on all of the cancer types under study in the VDPP; there was no specific number of cases that a study’s investigators were required to contribute, either overall or for a particular cancer type, to participate.

*Organizational structure and governance.* The VDPP Steering Committee was formed to oversee the design and execution of the VDPP. The Steering Committee consisted of the committee chair, investigators representing each of the participating cohorts, the head of the Data Coordinating Center, the lead laboratory investigator, and leaders of the working groups. Personnel from the NCI’s Division of Cancer Control and Population Sciences and the NCI’s Division of Cancer Epidemiology and Genetics also participated as ex-officio Steering Committee members. During the course of the study, the Steering Committee met 3 times in person at the annual NCI Cohort Consortium Conference in 2007, 2008, and 2009 and held monthly telephone conference calls. Decisions were generally made by consensus, but when voting was conducted, only representatives of the participating cohorts were designated voting members, with 1 vote each.

*Working groups.* Working groups were established to direct data analyses and write manuscripts for each cancer site and an analysis of correlates of 25(OH)D concentrations. Esophageal and gastric cancers were combined into 1 working group (upper gastrointestinal tract cancers); other cancer sites each had a working group. Working group leaders were chosen on a volunteer basis. The groups consisted of representatives from each cohort study, except when the cohort investigators were not providing serum samples or data for the specific cancer site. The leader of each working group was represented at the Steering Committee meetings. In addition, statistical and quality control working groups were created to develop the general statistical analysis plan and quality control procedures. Working groups communicated on a regular basis by e-mail or conference call.

*Data Coordinating Center.* All data for the VDPP were maintained at the Data Coordinating Center led by the

**Table 1.** Characteristics of Cohorts Included in the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers, 1974–2006

Cohort Study (Reference No.(s))	Population	Geographic Location	Size of Cohort With Blood Specimens		Dates of Blood Draw	Type of Specimen Collected	Case Ascertainment	Date of Last Follow-up
			No.	% Female				
ATBC (22, 23)	Smokers	Finland	29,133	0	1985–1988	Serum	Finnish Cancer Registry	April 2005 <sup>a</sup>
CPS-II (24)	General	United States—national	39,380	56	1998–2001	Serum	Cancer registry/medical records/NDI	December 2004
CLUE I/CLUE II (25)	General	United States—Washington County, Maryland	CLUE I: 3,165	59	CLUE I: 1974	Serum/ plasma	State and county cancer registries	December 2007
			CLUE II: 24,646	58	CLUE II: 1989			
HPFS (26)	Health professionals	United States—national	18,225	0	1993–1995	Plasma	Medical records/NDI	2006
MEC (27)	General	United States—Hawaii and California	67,594	54	2001–2006	Serum	SEER Registry	December 2006
NYU-WHS (28)	Mammography screenees	United States—New York, New York	14,274	100	1985–1991	Serum	Questionnaire/New Jersey, New York, and Florida cancer registries/NDI	July 2003
NHS (29–31)	Registered nurses	United States—national	32,826	100	1989–1990	Plasma	Medical records/NDI	2004
PLCO (32, 33)	General	United States—national	66,000	50	1993–2001	Serum	Questionnaire/medical records	October 2005
SMHS (34)	General	Shanghai, China	16,119	0	2001–2006	Plasma	Cancer registry	August 2008
SWHS (35)	General	Shanghai, China	56,831	100	1997–2000	Plasma	Cancer registry	October 2008

Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CPS-II, Cancer Prevention Study II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; MEC, Multiethnic Cohort Study; NDI, National Death Index; NHS, Nurses' Health Study; NYU-WHS, New York University Women's Health Study; 25(OH)D, 25-hydroxyvitamin D; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SEER, Surveillance, Epidemiology, and End Results; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study.

<sup>a</sup> April 2004 for pancreatic cancer and non-Hodgkin lymphoma.

**Table 2.** Characteristics of Participants in the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers, 1974–2006

Cohort	No. of Cases <sup>a</sup>	No. of Controls <sup>a</sup>	Race/Ethnicity Among VDPP Controls, %				Median Age (IQR <sup>b</sup> ) of VDPP Controls, years	Median Follow-up Time (IQR) for VDPP Cases, years
			White	Black	Asian	Other		
ATBC	1,223	1,747	100	0	0	0	58 (54–62)	8.7 (4.9–12.7)
CPS-II	376	382	98	<1	<1	1	70 (67–74)	2.3 (1.3–3.6)
CLUE	843	859	99	1	0	0	55 (47–64)	10.1 (5.3–14.7)
HPFS <sup>c</sup>	133	147	94	0	1	2	67 (59–72)	4.4 (2.6–6.7)
MEC	408	413	13	24	26	37 <sup>d</sup>	70 (63–75)	2.1 (1.1–3.3)
NYU-WHS	441	469	72	11	<1	4	55 (48–60)	10.8 (6.0–14.6)
NHS	435	851	99	<1	<1	<1	57 (52–63)	7.0 (4.0–9.5)
PLCO	950	1,141	92	4	4	<1	65 (61–68)	4.5 (2.2–6.8)
SMHS	198	202	0	0	100	0	66 (55–70)	1.7 (0.9–2.7)
SWHS	484	503	0	0	100	0	59 (49–65)	4.7 (2.4–6.6)

Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CPS-II, Cancer Prevention Study II Nutrition Cohort; IQR, interquartile range; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; NYU-WHS, New York University Women's Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study; VDPP, Cohort Consortium Vitamin D Pooling Project of Rarer Cancers.

<sup>a</sup> Includes cases and controls for certain cancer sites from the ATBC, NHS, and PLCO cohorts with previously measured 25-hydroxyvitamin D levels; numbers reflect those cases and controls included in the site-specific analyses (after exclusions described in the site-specific manuscripts).

<sup>b</sup> 25th–75th percentiles.

<sup>c</sup> For the HPFS, 3% of cohort participants were missing information on race/ethnicity.

<sup>d</sup> Includes Hispanics not categorized as being of "white" or "black" race/ethnicity.

Nutritional Epidemiology Branch of the NCI's Division of Cancer Epidemiology and Genetics. Information Management Services, Inc. (Silver Spring, Maryland) served as the data collection, database creation/storage, editing, and analytical unit. A secure portal for uploading of data from individual cohorts and the laboratory was established by Westat, Inc. (Rockville, Maryland), with restricted access.

**Approvals needed.** Investigators in each cohort study requested and received institutional review board approval from their home institution(s) to participate in the VDPP. In addition, the investigators for each cohort signed a data transfer agreement with the NCI governing the approved use of the data.

## Study design and methods

**Case and control definitions.** Cases included all persons with incident primary endometrial, kidney, ovarian, pancreatic, or upper gastrointestinal tract cancer or lymphoma who had plasma or serum samples available (Table 3). Histologic confirmation was not required for inclusion of a case in the VDPP; however, approximately 95% of the included cases were histologically confirmed (endometrial, 98.4%; kidney, 93.3%; lymphoma, 98.1%; ovarian, 96.3%; pancreatic, 82.0%; and upper gastrointestinal, 98.4%). In general, a single control was matched to each case on age at blood collection ( $\pm 1$  year), sex, race/ethnicity (white/black/Asian/other), and date of blood collection ( $\pm 30$  days if possible). Because, in some cases, cohort investigators may have previously selected case-control sets for prior studies, cases and

controls may have been matched on additional criteria, such as fasting status or menopausal status at the time of blood draw; in addition, the matching criteria for the VDPP matching variables may have differed (e.g., the matching criterion for age at blood collection may have been  $\pm 2$  years instead of  $\pm 1$  year). In particular, cases and controls in the New York University Women's Health Study (NYU-WHS) were matched on the date of blood collection by  $\pm 90$  days. In some instances, more than 1 control was matched to each case.

To be eligible, controls had to be alive and free of cancer (except possibly nonmelanoma skin cancer or cervical cancer in situ) and to possess the organ under study (e.g., uterus, ovary), if this information was available, on the calendar date on which their matched case's cancer was diagnosed. In total, 5,743 cases and 6,807 controls were initially identified for inclusion in the VDPP. After exclusions, which are described in each site-specific manuscript, data from a total of 5,491 cases and 6,714 matched controls were analyzed (Table 3). The lymphoma working group restricted their analyses to non-Hodgkin lymphoma, excluding other types of lymphoma.

**Vitamin D assays. Serum/plasma requirements.** All samples selected specifically for 25(OH)D measurement as part of the VDPP were assayed at Heartland Assays, Inc. (Ames, Iowa). For each case and control, 125  $\mu$ L of either serum or plasma was sent clot-free in a bar-coded screw-cap microcentrifuge tube (Sarstedt AG & Company, Nümbrecht, Germany). Published data indicate that there was no impact of type of specimen (serum or plasma) on 25(OH)D results

**Table 3.** Numbers of Cancer Cases Contributed By Each Cohort Participating in the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers, by Cancer Type, 1974–2006<sup>a</sup>

Cancer Site	ATBC	CPS-II	CLUE	HPFS	MEC	NHS	NYU-WHS	PLCO	SMHS	SWHS	Total
Endometrial	0	51	192	0	39	163	139	147	0	99	830
Kidney	286	58	102	0	64	0	35	161	32	37	775
Lymphoma <sup>b</sup>	208	135	236	133	96	145	73	286	8	33	1,353
Ovarian	0	27	102	0	18	127	94	74	0	74	516
Pancreatic	313	65	123	0	109	0	73	183	27	59	952
Upper gastrointestinal tract (esophagus and stomach)	416	40	88	0	82	0	27	99	131	182	1,065
Total	1,223	376	843	133	408	435	441	950	198	484	5,491

Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CPS-II, Cancer Prevention Study II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; NYU-WHS, New York University Women's Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study.

<sup>a</sup> Totals include samples that were assayed for 25-hydroxyvitamin D levels prior to initiation of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (189 ATBC lymphoma cases, 200 ATBC pancreatic cancer cases, 127 NHS ovarian cancer cases, and 183 PLCO pancreatic cancer cases and matched controls).

<sup>b</sup> Lymphoma cases that were not cases of non-Hodgkin lymphoma were excluded prior to analysis and therefore are not included in the numbers presented in the table. The numbers represent only cases of non-Hodgkin lymphoma.

(36, 37) and that there was no decline in 25(OH)D concentrations during 4 years of storage (38). Further, it has been shown that 25(OH)D results obtained for specimens that are thawed and refrozen up to 4 times are reliable (39), and therefore there was no restriction based on previous thaw/freezing cycles for the blood specimens. Measurement of 25(OH)D was conducted by means of a direct, competitive chemiluminescence immunoassay using the DiaSorin LIAISON 25(OH)D TOTAL assay (DiaSorin, Inc., Stillwater, Minnesota) (40). The assay is co-specific for 25-hydroxyvitamin D<sub>3</sub> and 25-hydroxyvitamin D<sub>2</sub>. The assay utilizes a specific antibody to 25(OH)D for coating magnetic particles (solid phase) and a vitamin D analog, 22-carboxy-23,24,25,26,27-pentanorvitamin D<sub>3</sub>, linked to an isoluminol derivative. During the incubation, 25(OH)D is dissociated from its binding protein and competes with the isoluminol-labeled analog for binding sites on the antibody. After the incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units and is inversely proportional to the concentration of 25(OH)D present in calibrators, controls, or samples.

**Quality control.** Several types of masked reference samples were interspersed among study samples for assessment of quality control. Investigators for each cohort were provided with samples of the vitamin D standard (standard reference material 972, Vitamin D in Human Serum) (41) from the US National Institute of Standards and Technology (NIST) at both level 1 (prepared from "normal" human serum and not altered) (~60 nmol/L) and level 2 (prepared by diluting level 1) (~35 nmol/L) to include with their samples. Every batch of 100 samples contained either 2 level-1 samples or 2 level-2 samples. In addition, masked quality control samples were provided by the investigators from each cohort, within each batch of their samples (either 2

or 4 cohort-specific quality control samples), in a manner that resulted in overall quality control (including the NIST samples) being 5% of the total sample. In general, the quality control samples provided by each cohort's investigators were either pooled samples from multiple subjects or specimens from separate individuals that were repeated throughout that cohort's batches.

Coefficients of variation for duplicate serum/plasma aliquots included in all laboratory sample batches were calculated for the 3 types of quality control samples: NIST level 1, NIST level 2, and each cohort quality control. The variance components model of SAS (PROC VARCOMP) was used to estimate the variance between batches and within batches and to calculate interbatch and intrabatch coefficients of variation (SAS Institute Inc., Cary, North Carolina). Inter- and intrabatch coefficients of variation for NIST level-1 samples were 12.7% and 9.3%, respectively; inter- and intrabatch coefficients of variation for NIST level-2 samples were 13.6% and 11.0%, respectively. The median interbatch coefficient of variation for the cohort quality control samples was 13.2% (range, 4.8%–17.0%); the median intrabatch coefficient of variation for the cohort quality control samples was 9.9% (range, 3.8%–16.4%).

**Covariate information.** A core set of covariates was selected by the Steering Committee to be considered in the statistical analyses of vitamin D and all of the rarer cancer sites. These covariates were selected on the basis of previously published data on their associations with vitamin D concentration and/or cancer in general and included demographic characteristics, family history of cancer, anthropometric factors, physical activity, alcohol use, supplement use, medication use, smoking habits, hormone therapy, oral contraceptive use, reproductive factors, sun exposure, history of certain diseases, and death data. Additional data on dietary intake (especially sources of vitamin D and dairy

foods), as well as hair and eye color, were collected for an analysis on correlates of circulating 25(OH)D concentration. Each working group was then asked to submit a limited “wish list” of additional variables for which data would be requested from the cohort studies’ investigators to be used in that working group’s analysis.

After the complete list of variables was compiled from the working groups, cohort investigators were asked to provide information about the availability of data on the variables and the time point of data collection (e.g., at the time of blood draw; prior to or after blood draw). For most variables, information collected at the time of blood draw or closest to the time of blood draw was used. A requested variable was removed from the core data request list if data were available from fewer than 4 cohorts. Working groups were then asked to reexamine their list of desired variables and revise it accordingly. For example, a working group may have requested data on occupational history; these data were available from only 3 of the cohorts and thus would have had limited usefulness in the analyses.

The Steering Committee reviewed the final variable list, and the Data Coordinating Center sent a single, complete data request form to investigators in each of the cohorts. This request included the name of each specific variable, the time point at which data for the variable were being requested (either the date of blood draw or the index date (date of the case’s diagnosis for both case and control(s) in a matched set)), and the desired format for the variable. The Data Coordinating Center cleaned and harmonized the data on all variables. The decision was made not to impute missing values; rather, missing values for each variable were coded as a unique category so that cases and controls were not dropped from the regression analyses when data were missing.

Latitude is a variable of specific interest in investigations of vitamin D. With the exception of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC), the Cancer Prevention Study Nutrition Cohort (CPS-II), the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), the Shanghai Men’s Health Study, and the Shanghai Women’s Health Study, latitude was determined using the first 3 digits of the subject’s zip code. The ATBC and Shanghai Men’s and Women’s Health Study subjects were assigned the latitudes of 60°N (the latitude of Helsinki, Finland) and 31°N (the latitude of Shanghai, China), respectively. PLCO subjects were assigned latitudes on the basis of the location of their local screening center. The CPS-II investigators provided data on latitude. For stratified analyses, latitude was categorized as <34°N, 34°N–42°N, and >42°N. These cutpoints were based on data published by Webb et al. (42) showing that at 42°N, cutaneous production of vitamin D ceases from November through February because of the angle of the sun and resulting decreases in the amount of ultraviolet B radiation that reaches the earth’s surface and that at 34°N, vitamin D is synthesized (although at low levels) all winter.

*Statistical approaches.* The strengths of the multisite cohort consortium approach also present challenges in terms of the data analyses. A clear strength of the NCI Cohort Consortium is the heterogeneity of regions and the diversity

of populations. This Cohort Consortium study included a cohort from Finland, a population that has low vitamin D exposure based on region, as well as cohorts comprised of Asian and African-American populations, which also have low vitamin D exposure because of skin pigmentation. Thus, the highest percentile of circulating 25(OH)D concentrations in these populations fell into the low-to-middle ranges of concentrations in other populations. To avoid masking any associations and to take advantage of the range of circulating 25(OH)D concentrations, we used multiple analytic approaches, including traditional pooling approaches and meta-analysis modeling.

The decision was made to conduct the main analyses for each cancer type using clinically defined cutpoints (43–45); the cutpoints used were <25, 25–<37.5, 37.5–<50, 50–<75, 75–<100, and ≥100 nmol/L. The referent category chosen was 50–<75 nmol/L, because this concentration includes the mean concentration of the US population (62.91 nmol/L (standard error, 0.81) for males and 61.54 nmol/L (standard error, 0.85) for females), based on 2000–2004 National Health and Nutrition Examination Survey data (46). Using these cutpoints, conditional logistic regression analysis was used to examine the association between 25(OH)D at high and low concentrations and the development of cancer. For some analyses (e.g., stratified analyses) for the individual cancer sites, the 2 highest categories were combined because of insufficient numbers in the highest category of circulating 25(OH)D. Tests for a trend in the categorical 25(OH)D variable in these (and other) analyses were performed using a Wald test, by assigning ordinal scores (0, 1, 2, 3, . . .) to each of the 25(OH)D categories and treating the variable as continuous in the regression model.

Concentrations of circulating 25(OH)D differ over the course of the year with variations in sun exposure and sun intensity. Cases and controls were matched within cohort and as closely as possible by date of blood draw. Overall, among controls measured for 25(OH)D as part of the VDP, 83.5% were matched within 30 days of the exact date of their matched case’s blood draw. Because matching within 30 days was not always possible, this requirement was relaxed for some matched pairs (5.9% of controls were matched within 60 days of the case and 3.3% were matched within 90 days of the case). Matching on month of blood draw, without regard to year, was also allowed, if needed. This was done for 110 controls, with the most extreme difference in the exact date of blood draw being 6.3 years. However, it is the month of the year that is most important for vitamin D assays, and 85.7% of cases and controls were matched within 30 days (regardless of year). In addition to matching, several other approaches were used in the analyses to deal with season: stratification of models by season, residual adjustment for season, and use of season-specific cutpoints.

Based on the distributions of circulating 25(OH)D data among controls, both overall and by cohort, 2-, 3-, and 4-category season variables were defined and tested. It was determined that the 2-season definition of “summer” (June–November) and “winter” (December–May) adequately characterized the seasonality differences, based on similar median circulating 25(OH)D concentrations by month,

while also maintaining large enough numbers in each subcategory. Therefore, all of the analyses discussed in the cancer-site-specific manuscripts were conducted using the 2-category season variable. Season-stratified analyses using the 2-category season variable were conducted for each cancer site using unconditional logistic regression, adjusting for matching factors. The 4-category season variable was used in the analysis of the correlates of 25(OH)D concentration, as described in the paper by McCullough et al. (47). The 4-season variable was categorized as: winter, January–March; spring, April–June; summer, July–September; and fall, October–December.

Use of regression residuals to adjust for season takes into account the gradual nature of changes in concentrations of 25(OH)D over the year, which may be better than adjusting for season. For residual adjustment for season, the residuals of circulating 25(OH)D concentrations (log-transformed) against week of blood draw were calculated using the local polynomial regression method (48). This adjustment was based on data from controls for all tumor sites within sex- and cohort-specific groups (with previously assayed samples, such as those from the ATBC Study, done separately) and then applied to all cases within those specific groups. The residual data were cut into sex- and cohort-specific quartiles based on the control distribution, and then all residual data were merged together. Conditional logistic regression analyses were conducted with the lowest quartile designated the referent category.

For models with season-specific cutpoints, pooled analyses were conducted using cohort-, sex-, and season-specific quartile cutpoints (using the 2-season variable), based on the distribution of circulating 25(OH)D concentrations among all control subjects from all cancer sites combined. This allowed for greater stability and resulted in the use of the same cutpoint definition across all cancer sites. A second set of quartile categories was created that were not cohort-specific, whereby cohorts with lower circulating 25(OH)D values fell mainly into the lower quartiles. Results obtained for each cancer site were similar for the first (cohort-specific) and second (not cohort-specific) sets of categories; thus, only results for the cohort-specific cutpoints are presented in the site-specific manuscripts.

Finally, using the a priori clinically defined cutpoints, a 2-stage meta-analysis based on the methods described by Smith-Warner et al. (49) was conducted. Results for each cancer type are presented for high concentrations of 25(OH)D (defined as either  $\geq 100$  nmol/L or  $\geq 75$  nmol/L, depending on the number of cases for a specific cancer type) versus the reference category (50– $<75$  nmol/L) and for low concentrations of 25(OH)D (defined as either  $<25$  nmol/L or  $<37.5$  nmol/L) versus the reference category. Cohort-specific odds ratios were estimated and then pooled using inverse-variance weights in random-effects models. The heterogeneity of cohort-specific estimates was measured using the DerSimonian and Laird  $Q$  statistic (50), and data are presented as forest plots.

**Stratified analyses.** Stratified analyses were conducted using conditional models for sex, race/ethnicity, and histologic subtype and unconditional models, adjusting for the matching factors, for variables such as season, age, and

body mass index. To examine latency effects, stratified analyses were also conducted for each cancer site based on time from blood draw to cancer diagnosis ( $\leq 5$  years vs.  $>5$  years and  $\leq 2$  years vs.  $>2$  years). In addition, each working group conducted stratified/subgroup analyses based on factors relevant to the specific tumor site.

Interaction tests were conducted for continuous variables by crossing the median values of each of the 25(OH)D categories with the median value (based on controls) of the stratified variable. The resulting continuous values were added to the model, and the  $P$  value for the interaction term was calculated. For categorical variables, multiple interaction terms were created, one for each level of the categorical variable crossed with the continuous variable, and the log-likelihood test was used to compare models with and without the interaction terms.

**Confounding.** The process used for identification of confounding factors was determined by the working group for each individual cancer site. Therefore, confounder identification is described in the Materials and Methods section of each site-specific manuscript.

**Inclusion of previous data.** For this study, 9,855 samples were assayed for 25(OH)D centrally for the VDPP and included in the site-specific analyses. In addition, investigators from some participating cohort studies contributed data from previously assayed samples (ATBC—non-Hodgkin lymphoma: 189 cases/506 controls; pancreatic cancer: 200 cases/400 controls; PLCO—pancreatic cancer: 183 cases/364 controls; Nurses' Health Study—ovarian cancer: 127 cases/381 controls). The distribution of circulating 25(OH)D concentrations among controls in the previously assayed samples was compared with the distribution of vitamin D concentrations from controls of the same cohort assayed as part of the VDPP; these samples were found to differ by an average of 31%, but the differences were not systematic when examined by cohort, race/ethnicity, age, and season. Therefore, no adjustments or calibration of the vitamin D data were performed, and the raw data from previously assayed samples were added to the categories of clinically defined cutpoints. Note that the calculation of cohort-, sex-, and season-specific quartiles, described above, was conducted separately for the previous data. Sensitivity analyses were conducted in these site-specific analyses after excluding these previously analyzed cases and controls; data from previously assayed samples were not included in the correlates analysis.

## VITAMIN D DATA

Table 4 shows mean values, median values, and interquartile ranges for circulating 25(OH)D concentrations measured as part of the VDPP (and not including the previous data) among controls, overall and by cohort. Among the controls of the participating cohorts, the Health Professionals Follow-up Study had the highest mean circulating 25(OH)D concentrations, and CPS-II had the highest median circulating 25(OH)D concentrations. The ATBC Study had the lowest mean and median circulating 25(OH)D concentrations. None of the circulating 25(OH)D measurements from samples collected within the Shanghai Women's

**Table 4.** Circulating 25-Hydroxyvitamin D Concentrations (nmol/L) Measured Among Controls in the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers, Overall and By Cohort, 1974–2006<sup>a</sup>

Cohort	Mean (SD)	Minimum	25th Percentile	Median	75th Percentile	Maximum
ATBC	35.8 (20.5)	2.4	20.3	31.8	48.4	145.6
CPS-II	61.6 (23.0)	16.6	46.0	59.7	74.4	143.0
CLUE	61.2 (25.0)	5.7	44.3	58.8	74.7	189.9
HPFS	61.8 (22.4)	10.0	45.8	58.6	76.6	138.7
MEC	53.1 (24.6)	5.0	35.3	50.2	66.6	146.3
NYU-WHS	48.7 (22.0)	6.4	32.8	46.4	62.1	140.9
NHS	56.8 (22.6)	9.9	41.1	53.8	69.4	145.4
PLCO	55.1 (21.6)	10.2	40.3	53.6	66.7	188.8
SMHS	40.9 (17.7)	9.2	28.2	38.0	50.3	106.8
SWHS	36.6 (15.7)	2.9	25.0	33.4	44.5	98.7
Total	50.6 (24.0)	2.4	33.1	48.1	64.4	189.9

Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CPS-II, Cancer Prevention Study II Nutrition Cohort; HPFS, Health Professionals Follow-up Study; MEC, Multiethnic Cohort Study; NHS, Nurses' Health Study; NYU-WHS, New York University Women's Health Study; PLCO, Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial; SD, standard deviation; SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study.

<sup>a</sup> Previously assayed samples were not included.

Health Study was above 100 nmol/L. Overall, the mean and median 25(OH)D concentrations among controls in the VDPP were 50.6 nmol/L and 48.1 nmol/L, respectively. The distribution of 25(OH)D concentrations among the controls was right-skewed.

## SUMMARY AND CHALLENGES

The potential role of vitamin D in health should be well understood so the public can be informed of both the benefits and potential risks of increasing vitamin D concentrations through supplementation or sun exposure. Recently, a working group of the International Agency for Research on Cancer (IARC) published a monograph calling for more extensive clinical trials to evaluate the health effects of vitamin D supplementation prior to instituting supplementation recommendations (5). Randomized clinical trials are critical, but such trials are expensive, focus on a specific dose, examine effects that occur late in disease progression, deal by nature with selected populations, and are not practical for examining rare outcomes with long latency periods such as the rarer cancers examined here. Well-conducted observational studies, though discounted in value by the IARC report, are complementary to clinical trials and in many cases will provide the only obtainable evidence of benefit or harm associated with specific exposures. Long-term prospective studies of circulating vitamin D concentrations can examine a range of exposure levels, can assess long-term health outcomes in populations closer in terms of characteristics to the general population than those included in clinical trials, and may cost-effectively examine the association between dose and multiple rarer outcomes. The VDPP provides a unique contribution to understanding the association between vitamin D concentrations resulting

from a wide range of vitamin D exposures and subsequent development of rarer cancers, overcoming many of the limitations of ecologic and case-control studies. Thus, this type of study provides complementary data to those which may be obtained from randomized clinical chemoprevention trials.

One of the principal strengths of the VDPP is the number of cases of rarer cancers included; these case numbers, because of the contribution of 10 cohorts, exceed those of other, single-site observational studies and allow for rigorous analysis of the associations examined. Further, the cohorts in the VDPP have a wide geographic distribution, covering extremes in latitude and thus sun exposure. This variation in latitude and sun exposure permits the estimation of cancer risks at very high and low vitamin D concentrations, in contrast to single-site observational studies, which may have a limited range of vitamin D values. Other strengths of the VDPP were the availability of prospectively collected blood samples for measurement of 25(OH)D as an integrated biomarker of vitamin D exposure (instead of examining vitamin D using dietary information and/or sun exposure data only); the use of a centralized laboratory to perform the vitamin D assays, which allowed for the standardization of vitamin D assay methods and instrument calibration; and the availability of the NIST standards for quality control.

Despite the strengths of the VDPP, there were a number of inherent challenges to the project that arose primarily from the same source as its strengths. Unlike clinical trials, in which common data elements are determined and collected specifically for the study, this project included existing cohorts that had diverse methods of data collection. Thus, data collection and harmonization of data elements were challenging, and some variables that may have been



confounders could not be included in the analyses. Handling of missing data was also a challenge, and ultimately a decision was made to not impute data. Further, despite the large sample size overall for each cancer site in comparison with previous studies, the numbers were small when the analyses were stratified on certain variables.

An often-cited limitation of serologic studies is the reliance on a single 25(OH)D measurement per subject. Substantial variation in 25(OH)D concentrations over time has been shown in a number of published studies (5, 43), and 25(OH)D status at a single time point may not accurately reflect a person's long-term 25(OH)D exposure. However, the NYU-WHS investigators conducted a pilot study to assess the temporal reliability of 25(OH)D values using 3 samples collected at yearly intervals from 30 healthy NYU-WHS participants (15 premenopausal and 15 postmenopausal) and found that temporal reliability, estimated using the intraclass correlation coefficient, was excellent (intraclass correlation coefficient = 0.78, 95% confidence interval: 0.64, 0.88) (A. Zeleniuch-Jacquotte, New York University School of Medicine, personal communication, 2009). Similarly, an analysis using data obtained in the Nurses' Health Study showed an intraclass correlation coefficient of 0.72 (95% confidence interval: 0.62, 0.80) for 25(OH)D concentrations measured in 71 women over a period of 2–3 years (S. E. Hankinson, Harvard School of Public Health, personal communication, 2009). This result is comparable to that observed in 144 middle-aged men, for whom the correlation between samples collected 3 years apart was 0.70 (51).

Seasonal variation in vitamin D concentrations is another potential limitation to serologic studies and is probably more important at higher latitudes than at lower latitudes because of the wide variations in sun elevation angles with increasing latitude (5). The long-term intraindividual variation of circulating 25(OH)D concentrations across seasons has not been well studied and thus is not well understood. Nakamura et al. (52) reported a Pearson correlation coefficient of 0.46 ( $P < 0.0001$ ) for correlation between circulating concentrations of 25-hydroxyvitamin D<sub>3</sub>, a major component of 25(OH)D, measured in the summer and the fall among 122 healthy Japanese women aged 41–81 years. In this study, to minimize bias from seasonal variation, a number of strategies were employed in both the design of the study and the analytic methods, including matching on the date of blood draw, using season-specific cutpoints to create exposure categories, stratifying on season, and conducting residual-adjusted analyses. In general, the results from these analyses were consistent for all cancer sites; however, the possibility of bias due to inaccurate classification of long-term 25(OH)D exposure cannot be ruled out.

Despite the claim by the IARC vitamin D working group that “new observational studies are unlikely to disentangle the complex relationships between vitamin D and known cancer risk factors” (5, p. 1), consortial approaches such as the VDPP are complementary to clinical trials, addressing aspects that may only be answered by long-term observational studies, particularly for determining associations with rare cancer outcomes. Study designs are complementary, since no one study and no one type of study design are likely to be sufficient to answer these types of questions. The

VDPP represents one step towards understanding the relation between vitamin D and the development of rarer cancers.

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