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Annals of Oncology 24: 687–692, 2013
doi:10.1093/annonc/mds509
Published online 7 November 2012

Prospective analysis of vitamin D and endometrial cancer risk

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Received 25 June 2012; revised 10 August 2012; accepted 17 August 2012

Background: This is the first prospective cohort analysis on the association between vitamin D and endometrial cancer incorporating time-varying predicted plasma 25-hydroxyvitamin D [25(OH)D].
Methods: The prospective cohort analysis of predicted 25(OH)D and total dietary vitamin D intake used the Cox proportional hazards model, and involved 644 incident endometrial cancer events from 1986 to 2006 in the Nurses' Health Study. Genotyping and unconditional logistic regression were carried out on 572 endometrial cancer cases and their matched controls on 12 single nucleotide polymorphisms (SNPs) in vitamin D-related genes.
Results: There was no significant association between predicted 25(OH)D and endometrial cancer incidence, with the hazard ratio for the highest (versus the lowest) quintile of predicted 25(OH)D as 1.00 (95% CI 0.73–1.36) (p-trend = 0.33). There was also no significant association involving total dietary vitamin D. No significant associations between any of the vitamin D-related SNPs and endometrial cancer were observed.
Conclusion: Both predicted 25(OH)D and total dietary vitamin D intake were not associated with endometrial cancer incidence. These results suggest that vitamin D may not protect against the development of endometrial cancer. However, the low and narrow vitamin D exposure range in the cohort may limit generalizability of the results.
Key words: endometrial cancer, epidemiology, vitamin D

introduction

There is substantial interest in the anticancer role of vitamin D, a nutrient traditionally associated with calcium metabolism. Vitamin D is synthesized following the skin's exposure to solar ultraviolet B (UVB) radiation, is found naturally in foods such as fish liver oil and fatty fish species, and is fortified in foods such as milk and cereal. Vitamin D and its metabolites are primarily transported by the vitamin D-binding protein in the circulation. Two hydroxylations allow vitamin D to become biologically active. The first hydroxylation occurs in the liver to

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convert vitamin D to 25-hydroxyvitamin D [25(OH)D]. The second hydroxylation occurs in various organs to form the physiologically active 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], which binds to the vitamin D receptor (VDR) in the cell nucleus. There is strong biological plausibility that vitamin D has an anticancer role. Cells in various parts of the body not involved with calcium metabolism contain VDRs and synthesize 1,25$(OH)_2$D locally; for these different cell types, 1,25$(OH)_2$D inhibits proliferation and promotes differentiation [1–3]. There is also epidemiological evidence, although not entirely consistent, supporting the protective role of vitamin D against the development of colorectal, breast, ovarian, prostate, and other cancers [4, 5].

Endometrial cancer is the most common cancer of the female reproductive system and the fourth most common cancer in women [6]. Most endometrial cancers are adenocarcinomas that originate from the glandular epithelial tissue that line the endometrium. Risk factors for endometrial cancer include older age, family history, estrogen only hormone replacement therapy, obesity, nulliparity, early menarche, late menopause, diabetes, radiation therapy, and Tamoxifen [7]. Expression of the VDR and the enzyme involved in 1,25$(OH)_2$D synthesis has been detected in human endometrial tissue [8–10].

Few epidemiological studies have examined the potential effect of vitamin D on endometrial cancer, and there has been no prospective cohort analysis incorporating multiple vitamin D measures over time. An ecological study found an inverse association between UVB irradiance and endometrial cancer incidence [11]. Three hospital-based case–control studies examined the association between dietary vitamin D and endometrial cancer risk [12–14], and a meta-analysis of these studies showed high between-study heterogeneity and an overall null association [15]. A recent pooled study using circulating 25(OH)D concentrations measured at a single time point found no association with endometrial cancer incidence [16]. This nested case–control study involved 830 cases and 992 controls from seven cohorts, and found no evidence of trend after adjusting for cohort, age, race, season at blood draw, and body mass index (BMI).

The main purpose of this analysis is to evaluate the association between vitamin D and endometrial cancer incidence by using predicted 25(OH)D updated biennially over 20 years of follow-up in the Nurses' Health Study (NHS). The 25(OH)D prediction method [17, 18] is used to estimate 25 (OH)D at multiple time points, using prospectively collected information on vitamin D determinants. This analysis also examines for the first time the associations between vitamin D-related single nucleotide polymorphisms (SNPs) and endometrial cancer, as well as the interaction between predicted vitamin D and these gene variants.

methods

study population

The NHS is a prospective cohort study that began in 1976, when 121 700 female registered nurses aged 30–55 years and residing in 11 US states completed an initial questionnaire. Personal information such as lifestyle and dietary factors was subsequently updated every 2 or 4 years through questionnaire responses. From 1989–1990, blood was collected from 32 826 participants. About 97% of these blood samples arrived within 26 h of being drawn and were centrifuged and aliquotted into plasma, white blood cell, and red blood cell components.

For the cohort analysis, nurses who had hysterectomy, surgical menopause, or cancers other than nonmelanoma skin cancer were excluded at baseline (defined as 1986, the first year of predicted 25(OH)D derivation) and each subsequent follow-up cycle. There were 946 264 person-years of data involving 644 cases in the cohort analysis, covering the timeframe from 1986 to 2006.

For the nested case–control analysis, genotyping was carried out on 572 cases and their matched controls who were selected from the NHS cohort. Controls were randomly selected up to and including the questionnaire cycle in which the case was diagnosed. Matching factors for cases and controls include age, menopausal status, date, and postmenopausal hormone use at specimen collection, and fasting status at blood collection. The NHS protocol was approved by the Human Research Committee of the Brigham and Women's Hospital, Boston, MA.

vitamin D exposure assessment

Vitamin D exposure was quantified as predicted plasma 25(OH)D derived biennially from 1986 to 2004. The plasma 25(OH)D prediction model was originally developed by Giovannucci et al. [17] for the Health Professionals Follow-up Study. In the NHS, the plasma 25(OH)D prediction model was developed from 2079 women with a single plasma 25(OH)D measurement from June 1989 to March 1991 [18]. The prediction method uses a linear regression model with plasma 25(OH)D as the outcome and the following covariates: age, vitamin D intake from food, vitamin D intake from supplements, UVB flux based on state of residence, physical activity, race, BMI, alcohol intake, postmenopausal hormone use, laboratory batch, and season of blood draw. Age, laboratory batch, and season of blood draw were controlled for in the model, but not used in the derivation of the predicted 25(OH)D [18]. The estimated regression coefficients were used to calculate the predicted plasma 25(OH)D in all NHS participants. Analyses involving the most recent and cumulative average predicted 25(OH)D gave similar results, so the cumulative average results were presented. An example of the derivation and use of the cumulative average exposure level is that the predicted 25(OH)D at 1986 was used as the vitamin D exposure level for the 1986 to 1988 timeframe, whereas the average of the predicted 25(OH)D at 1986 and 1988 was used as the exposure level for the 1988 to 1990 timeframe, and so on.

endometrial cancer case ascertainment

Cases of invasive type 1 endometrioid adenocarcinoma, diagnosed between 1986 and 2006, were confirmed by medical record review. There were 644 incident endometrial cancer cases during this time period who did not have missing predicted 25(OH)D data and were not excluded by the exclusion criteria. Among those cases, 572 had blood or buccal cell samples from which DNA was extracted for genotyping.

questionnaire information

Questionnaire information was obtained from the follow-up cycles. Information on potential confounders was updated every 2 years when available. Updated BMI was calculated using height reported at baseline and weight reported at each cycle. Those missing weight in one cycle had their weight carried forward from the previous cycle, while those missing weight for two consecutive cycles were excluded from the analysis until they again reported their weight. Smoking was quantified using pack-years. Information on dietary sources of nutrients was obtained from a food

frequency questionnaire (FFQ) that has been updated every 4 years since 1986. Dietary intake level was then calculated using the FFQ information as well as data from the US Department of Agriculture [19, 20]. Dietary sources include both natural food and dietary supplements. The cumulative average dietary intake level was used, adjusted for total energy intake.

SNP selection and genotyping

The 12 SNPs of interest were either selected from the *VDR* and vitamin D-binding protein (*Gc*) genes, or from a recent genome-wide association study (GWAS) meta-analysis on genetic predictors of circulating vitamin D levels [21]. *VDR* is on chromosome 12q13 and *Gc* is on chromosome 4q11-13. The selected *VDR* SNPs have been studied in relationship to various cancers other than that of the endometrium [22]. These *VDR* SNPs are *Fok1* (rs2228570), *Cdx2* (rs11568820), *VDR-5132* (rs1989969), *Bsm1* (rs1544410), *Apa1* (rs7975232), *Taq1* (rs731236), and *Bgl1* (rs739837). SNPs on the *Gc* are rs4588 and rs7041. The remaining three SNPs from the GWAS meta-analysis are rs1790349, rs6599638, and rs2060793, which are, respectively, on the *DHCR7*, *C10orf88*, and *CYP2R1* genes. The minor allele frequencies (MAF) range from 0.16 to 0.48. Genomic DNA was extracted from blood and buccal samples using the QIAmp (Qiagen, Chatsworth, CA) 96-spin blood protocol. Genotyping was carried out at the Dana Farber/Harvard Cancer Center High-Throughput Genotyping Core using the 5′ nuclease assay (Taqman, Applied Biosystems, Foster City, CA). Blinded quality control samples were inserted to validate genotyping procedures. Laboratory personnel were blinded to case–control status, and 5% blinded quality control samples were inserted to validate genotyping procedures; concordance for blinded samples was 100%. The amount of missing genotyping data was <4%.

statistical analyses

The Cox proportional hazards model was used in the cohort analysis. The model was stratified by calendar year (continuous) and age (continuous, months), and adjusted for smoking (0, 0.1–20, 20.1–40, and >40 pack-years), BMI (continuous, kg/m^2), race (White, Black, and others), age at menarche (7–11, 12, 13, and 14–18 years), oral contraceptive use (no use, <1, 1–3, 3–6, and >6 years), menopausal status (premenopausal and postmenopausal), postmenopausal hormone use (no use, oral conjugated estrogen, oral estrogen and progesterone, and others), and parity (0, 1, 2, 3, and >3). Predicted 25(OH)D was categorized in quintiles and the hazard ratio (HR) and 95% confidence intervals (CIs) were reported for risk of endometrial cancer for each quintile relative to the first quintile. Tests for linear trend involved ordering the quintiles of predicted 25(OH)D and using the resulting continuous variable in the multivariate model. The Anderson–Gill data structure was used to efficiently handle time-varying covariates [23].

The unconditional logistic regression model was used in the nested case–control analysis involving SNPs, and the study population was restricted to Caucasians. The additive genetic model was used, which assumes that the effect of the heterozygous genotype is intermediate between the two homozygous genotypes. The homozygous genotype of the reference allele was coded as 0. In the analysis of the association between vitamin D-related SNPs and endometrial cancer incidence, the matching factors age and menopausal status were adjusted in the model and the odds ratio (OR) was reported.

Analyses were done using the SAS Version 9.1 software (SAS Institute, Cary, NC). Quintiles were created using the rank procedure. Multiplicative interaction terms involving continuous variables were created to test for effect modification using the Wald test. All *P* values were two-sided.

results

Table 1 shows the descriptive characteristics of the study population at baseline in 1986, the first year when the predicted 25(OH)D can be reasonably derived. Women with lower predicted 25(OH)D had fewer pack-years of smoking, fewer months of oral estrogen use, higher BMI, and lower calcium, folate, and retinol intake than women with higher predicted 25(OH)D.

For the multivariate analyses of Tables 2 and 3, the quintile median values of the predicted 25(OH)D ranged from 24.1 to 32.2 ng/ml, whereas the quintile median values of the total dietary vitamin D ranged from 164.1 to 708.9 IU/day. The quintile median values of supplemental vitamin D intake ranged from 76.1 to 362.8 IU/day. The suggested guideline for 25(OH)D levels is that 20–29.9 ng/ml is vitamin D insufficient and ≥30 ng/ml is vitamin D sufficient [24]. However, the predicted 25(OH)D does not have the variability of the plasma 25(OH)D level because of the limited number of predictors that can be included in the prediction model. The vitamin D Dietary Reference Intake level of the National Academies Press for women 50–70 years old is 400 IU/day.

After adequately adjusting for potential confounders, there was no significant dose–response between predicted 25(OH)D and endometrial cancer incidence (p-trend = 0.33) (Table 2).

Table 1. Descriptive statistics for key variables in 1986 by quintiles of predicted 25(OH)D

Variables	Mean (SD)				
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Age (years)	52.5 (7.0)	51.5 (7.1)	51.4 (7.2)	52.0 (7.3)	53.5 (7.4)
BMI (kg/m^2)	29.7 (6.3)	25.2 (4.0)	24.4 (3.5)	23.6 (3.2)	22.9 (2.8)
Total dietary vitamin D (IU/day)	229.9 (183.9)	280.7 (207.8)	343.7 (232.1)	419.3 (265.7)	528.3 (282.1)
Total dietary folate (μg/day)	347.2 (179.6)	376.3 (200.8)	413.7 (222.4)	463.5 (245.0)	520.9 (260.3)
Total dietary retinol (IU/day)	2920.5 (3506.6)	3476.1 (4025.4)	4179.2 (4565.6)	5148.4 (4985.4)	6482.2 (5697.5)
Total dietary calcium (mg/day)	970.9 (488.6)	1055.8 (514.3)	1116.1 (529.0)	1193.2 (546.1)	1312.3 (570.2)
Oral estrogen use (months)	0.6 (6.7)	1.0 (8.3)	2.3 (13.3)	4.6 (19.8)	7.5 (22.7)
Parity (number of children)	3.0 (1.7)	3.0 (1.6)	3.0 (1.6)	2.9 (1.6)	2.8 (1.6)
Smoking (pack-years)	11.3 (17.2)	11.8 (17.1)	12.4 (17.5)	12.8 (17.9)	15.8 (20.2)
Age at menarche (years)	12.4 (1.4)	12.5 (1.4)	12.6 (1.4)	12.6 (1.4)	12.6 (1.4)
Oral contraceptive use (months)	22.3 (49.3)	25.3 (50.6)	27.3 (51.0)	27.8 (53.7)	30.2 (57.0)

Table 2. Multivariate cohort analysis of predicted 25(OH)D and endometrial cancer incidence

Predicted 25(OH)D	ng/ml[c]	NHS cohort		Model 1[a]		Model 2[b]	
		Events	Person-years	HR (95% CI)	β (*p*-trend)	HR (95% CI)	β (*p*-trend)
Q1 (reference)	24.1	214	203 497	—		—	
Q2	26.8	101	207 212	0.47 (0.37–0.60)	−0.12 (< 0.001)	0.71 (0.54–0.92)	0.04 (0.33)
Q3	28.6	112	196 756	0.56 (0.44–0.70)		0.91 (0.69–1.18)	
Q4	30.2	113	179 113	0.61 (0.48–0.76)		1.02 (0.77–1.36)	
Q5	32.2	104	159 686	0.56 (0.44–0.71)		1.00 (0.73–1.36)	

[a]Adjusted for calendar year (continuous) and age (continuous, months).
[b]Adjusted for calendar year (continuous), age (continuous, months), smoking (0, 0.1–20, 20.1–40, and >40 pack-years), BMI (continuous, kg/m^2), race (White, Black, and others), age at menarche (7–11, 12, 13, and 14–18 years), oral contraceptive use (no use, <1, 1–3, 3–6, and >6 years), menopausal status (premenopausal and postmenopausal), postmenopausal hormone use (no use, oral conjugated estrogen, oral estrogen and progesterone, and others), and parity (0, 1, 2, 3, and >3).
[c]Median values for each quintile.

Table 3. Multivariate cohort analysis of total dietary vitamin D and endometrial cancer incidence

Total dietary vitamin D	IU/day[c]	NHS cohort		Model 1[a]		Model 2[b]	
		Events	Person-years	HR (95% CI)	β (*p*-trend)	HR (95% CI)	β (*p*-trend)
Q1 (reference)	174.1	120	191 366	—		—	
Q2	308.7	131	195 996	1.02 (0.80–1.31)	0.01 (0.72)	1.00 (0.77–1.28)	0.01 (0.61)
Q3	430.5	139	195 709	1.08 (0.85–1.39)		1.06 (0.83–1.35)	
Q4	553.7	121	188 602	0.95 (0.74–1.23)		0.94 (0.73–1.22)	
Q5	729.1	133	174 591	1.09 (0.85–1.40)		1.10 (0.86–1.42)	

[a]Adjusted for calendar year (continuous) and age (continuous, months).
[b]Adjusted for calendar year (continuous), age (continuous, months), smoking (0, 0.1–20, 20.1–40, and >40 pack-years), BMI (continuous, kg/m^2), race (White, Black, and others), age at menarche (7–11, 12, 13, and 14–18 years), oral contraceptive use (no use, <1, 1–3, 3–6, and >6 years), menopausal status (premenopausal and postmenopausal), postmenopausal hormone use (no use, oral conjugated estrogen, oral estrogen and progesterone, and others), and parity (0, 1, 2, 3, and >3).
[c]Median values for each quintile. Supplemental vitamin D quintile median intake values are Q1: 76.1 IU/day, Q2: 155.2 IU/day, Q3: 228.0 IU/day, Q4: 290.3 IU/day, and Q5: 362.8 IU/day.

In the categorical analysis involving quintiles of predicted 25(OH)D, there were also no significant associations with endometrial cancer incidence, with the HR for the highest (versus the lowest) quintile of predicted 25(OH)D as 1.00 (95% CI 0.73–1.36). Similar results were observed in continuous and categorical analyses involving total dietary vitamin D (Table 3). Comparing the effect estimates obtained from Models 1 and 2 for Tables 2 and 3 indicated that the predicted 25(OH)D was more sensitive to confounding than the dietary vitamin D measurement, which highlighted the importance of adequately adjusting for confounding in association analyses involving the predicted 25(OH)D. None of the vitamin D-related SNPs were significantly associated with endometrial cancer (Table 4).

There was no evidence of effect modification of the association between predicted 25(OH)D and endometrial cancer by total dietary folate, total dietary retinol, total dietary calcium, and smoking. In addition, none of the vitamin D-related SNPs were significant effect modifiers of the predicted 25(OH)D and endometrial cancer association.

discussion

In this analysis, there was no association between vitamin D status and endometrial cancer incidence in the NHS study population. These findings are consistent with those from previous studies [15, 16].

This is the first study on endometrial cancer to use a prediction model for plasma 25(OH)D to quantify vitamin D exposure prospectively at multiple time points, whereas previous studies measured dietary vitamin D or circulating 25(OH)D levels at a single time point. The relevant anticancer dose of vitamin D might be best quantified by average long-term exposure level, in which case a single plasma 25(OH)D measurement may not adequately capture the vitamin D and cancer association. While circulating 25(OH)D level has been accepted as the best available biomarker for assessing vitamin D status [25, 26], it is often measured only once per participant due to the cost and inconvenience of obtaining multiple blood samples. In addition, studies that examined the correlation of 25(OH)D levels over time [27, 28] showed that the correlation of the 25(OH)D levels decreases as the time between their measurements increases. The lower correlation over a longer period of time may be explained by relocation in residence to a place with a significantly different solar UVB radiation level, or by lifestyle changes that influence vitamin D exposure level, such as reducing outdoor activity or starting vitamin D supplement intake [29]. The prediction model incorporates the above factors as well as information on other

Table 4. Associations between vitamin D-related SNPs and endometrial cancer incidence

SNP	Gene	MAF	Reference allele[a]	Endometrial cancer	
				OR (95% CI)[b]	*P*
rs6599638 (A,G)	*C10orf88*	0.48	A	1.13 (0.96–1.33)	0.15
rs2060793 (A,G)	*CYP2R1*	0.38	A	1.06 (0.89–1.27)	0.48
rs1790349 (C,T)	*DHCR7*	0.16	C	1.07 (0.85–1.36)	0.57
rs7041 (A,C)	*Gc*	0.41	A	0.95 (0.80–1.13)	0.54
rs4588 (G,T)	*Gc*	0.31	G	0.99 (0.82–1.20)	0.95
rs7975232 (A,C)	*VDR (Apa1)*	0.48	A	1.09 (0.92–1.28)	0.33
rs739837 (G,T)	*VDR (Bgl1)*	0.47	G	0.98 (0.84–1.15)	0.82
rs1544410 (G,A)	*VDR (Bsm1)*	0.40	G	1.00 (0.84–1.18)	0.98
rs11568820 (A,G)	*VDR (Cdx2)*	0.23	A	1.02 (0.83–1.24)	0.88
rs2228570 (A,G)	*VDR (Fok1)*	0.40	A	1.03 (0.87–1.23)	0.74
rs731236 (A,G)	*VDR (Taq1)*	0.40	A	1.00 (0.85–1.18)	0.97
rs1989969 (A,G)	*VDR (VDR-5132)*	0.40	A	1.04 (0.88–1.24)	0.62

[a]The homozygous genotype of the reference allele was coded as 0.
[b]Adjusted for the matching factors age and menopausal status.

lifestyle and dietary vitamin D determinants that is updated every 2 or 4 years using the NHS questionnaire. The frequent administration of questionnaires in the NHS makes it possible to predict 25(OH)D throughout the entire study follow-up, and these predicted levels collectively may be more indicative of long-term vitamin D status than plasma 25(OH)D measured at a single time point [18]. In addition to the above strength, the predicted 25(OH)D calculations are well-documented and have been applied to several studies [17, 30, 31]. Analyses of predicted and circulating 25(OH)D have already been done in separate studies for colorectal [30, 32] and pancreatic [31, 33] cancer. Therefore, this analysis using the predicted 25(OH)D complements the recently published pooled study involving circulating 25(OH)D and endometrial cancer incidence [16].

We also evaluated whether nutritional (folate, calcium, and retinol) and genetic factors (vitamin D-related SNPs) modified the association between vitamin D and endometrial cancer incidence, because of biological or epidemiological plausibility. There is biological evidence that the calcium and $1,25(OH)_2D$ signaling pathways interact in the growth control of cancer cells [34]. Epidemiological studies have also shown that vitamin D and calcium may interact to influence cancer risk [35, 36]. Folate may play a role in the epigenetic regulation of vitamin D hydroxylase expression [37]. Retinol intake may compete for retinoid X receptors and antagonize the actions of vitamin D [38], and an epidemiological study showed that high retinol intake countered the protective effect of vitamin D on distal colorectal adenoma risk [39]. Polymorphisms in genes for the VDR and other enzymes in the vitamin D activation pathway have been shown to modify the association between vitamin D and cancer risk [40]. None of these factors were found to be significant effect modifiers in this population; however, we had limited statistical power to detect interactions.

There are several limitations in this analysis. First, while the predicted 25(OH)D has potential advantages over a single blood measure, and incorporates multiple determinants of vitamin D status, there is still substantial unexplained variability in this exposure variable. For example, the UVB radiation exposure was assessed by an ecological variable that might not capture the individual exposure level. Therefore, in the 25(OH)D prediction model, we also included the physical activity variable, which is highly correlated with outdoor sun exposure. However, despite our effort to create a comprehensive prediction model, the predicted 25(OH)D measure may still be misclassified and consequently biased its association with endometrial cancer towards the null. Second, the plasma 25(OH)D outcome in the predicted 25(OH)D model may not reflect the endometrial tissue 25(OH)D level. Unfortunately, because it is impractical to sample tissues for 25(OH)D measurements in healthy controls, this limitation is present in all cancer epidemiological studies using plasma 25(OH)D. Third, the generalizability of the association between the predicted 25(OH)D and endometrial cancer incidence may be limited by the low and narrow range of vitamin D exposure. The majority of the women in the cohort had predicted 25(OH)D under 30 ng/ml, and the range of the median values of the lowest and highest quintiles of predicted 25(OH)D was 8.1 ng/ml. The protective benefits of vitamin D against endometrial cancer may not manifest unless 25(OH)D levels are significantly higher than 30 ng/ml and the range of exposure is much wider. Finally, because genotyping is only done in a sample of women in the cohort, statistical power is reduced in analyses involving vitamin D-related SNPs.

In conclusion, this prospective cohort analysis of the NHS population suggests that there is no association between vitamin D and endometrial cancer either using long-term average predicted 25(OH)D or total dietary vitamin D alone. It will be interesting for future studies to examine whether vitamin D plays a role in endometrial cancer progression and survival.

acknowledgements

We thank the NHS participants and staff for their contributions, as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY.

funding

This work was supported by the National Institutes of Health [P01 CA87969, R01 CA49449, and R01 CA082838]; the United States Army Medical Acquisition Activity [W81XWH-11-2-0131]; and the Nutritional Epidemiology of Cancer Education and Career Development Program [R25 CA098566 to K.A.B.].

disclosure

The authors have declared no conflicts of interest.

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