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Seasonal variation in expression of markers in the vitamin D pathway in prostate tissue

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Abstract

Purpose—Recent studies suggest variation in genes along the vitamin D pathway, as well as vitamin D receptor (VDR) protein levels, may be associated with prostate cancer. As serum vitamin D levels vary by season, we sought to determine whether expression of genes on the vitamin D pathway, assessed in prostate tumor tissue, do the same.

Methods—Our study incorporates mRNA expression data from 362 men in the Swedish Watchful Waiting cohort, diagnosed between 1977 and 1999, and 106 men enrolled in the US Physicians' Health Study (PHS) diagnosed between 1983 and 2004. We also assayed for VDR protein expression among 832 men in the PHS and Health Professionals Follow-up Study cohorts. Season was characterized by date of initial tissue specimen collection categorically and by average monthly ultraviolet radiation levels. One-way analysis of variance was used to examine variation in expression levels of six genes on the vitamin D pathway – VDR, GC, CYP27A1, CYP27B1, RXR α , CYP24A1 – and VDR protein by season, adjusted for age at diagnosis and Gleason grade. Variation was also examined separately among lethal and nonlethal cases.

Results—Tumor expression levels of the six genes did not significantly vary by season of tissue collection. No consistent patterns emerged from subgroup analyses by lethal versus nonlethal cases.

Conclusions—Unlike circulating levels of 25-OH vitamin D, expression levels of genes on the vitamin D pathway and VDR protein did not vary overall by season of tissue collection. Epidemiological analyses of vitamin D gene expression may not be biased by seasonality.

Keywords

Vitamin D; Vitamin D receptor; prostate cancer; gene expression; seasonality; biomarkers

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Conflict of Interest

The authors declare that they have no conflict of interest.

Introduction

Variation in genes along the vitamin D pathway, including those encoding vitamin D receptor (*VDR*) [1], 1 α -hydroxylase (*CYP27B1*), and 24-hydroxylase (*CYP24A1*) have been associated with prostate cancer progression in several epidemiological studies [2–4]. In experimental models of prostate cancer, 25-hydroxyvitamin D₃ (25(OH)D) is converted to the hormonally active 1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃) [5], which can inhibit proliferation, angiogenesis, and metastasis, and upregulate apoptosis in prostate cancer cells [6]. As ultraviolet (UV) radiation from sunlight is the major source of vitamin D, variations in the cutaneous synthesis [7] of precursor vitamin D₃, and thus serum levels of 25(OH)D [8–10], have been observed by season and geographical location. In the Nordic countries, nearly half the population had serum 25(OH)D below the recommended levels during winter months, although levels were satisfactory during summer months even at extreme Northern latitudes [11]. In the United States, considerable variation in cutaneous precursor vitamin D₃ synthesis exists between cities at northern and southern latitudes, corresponding to the availability of solar UVB radiation [7].

We previously showed that high VDR protein expression in prostate tumor tissue was associated with a reduced risk of lethal prostate cancer [12]. In the present study, we explore whether mRNA expression levels of genes in the vitamin D pathway as well as VDR protein expression in prostate tumor tissue vary seasonally, similar to serum vitamin D levels [10], as this may impact the interpretation of existing and future epidemiological analyses of these biomarkers. We hypothesized that VDR mRNA and protein expression may be highest in winter when circulating vitamin D levels are lower, while the other metabolizing genes may be expressed at higher levels during summer months, corresponding to higher serum vitamin D levels.

Methods

Study population

The study was based on two cohorts of prostate cancer patients from the United States and one from Sweden. mRNA expression of genes in the vitamin D pathways was available for two populations of men with prostate cancer nested within the Swedish Watchful Waiting cohort and the prospective US Physicians' Health Study. A subset of these men had available archival tumor tissue samples and was included in a gene expression profiling study to identify molecular signatures of lethal prostate cancer [13, 14]. Following the design of the original study, participants in our analysis included men who died from prostate cancer or developed bone metastases during follow-up (lethal disease), as well as those who survived 10 years after diagnosis (nonlethal disease). All cases included in this analysis had high-density tumor regions identified in tissue collected from diagnostic transurethral resection of the prostate (TURP) or radical prostatectomy (RP) samples [13]. Multiple 0.6mm cores were taken from high-density areas of formalin-fixed paraffin embedded tumor blocks, with RNA extracted from these cores using the CyBio-Well liquid handling system (CyBio AG, Jenna, Germany) [14]. More detailed methods have been published previously [13, 14].

Participants in the Swedish cohort were incidentally diagnosed with localized prostate cancer at University Hospital in Örebro (1977–94) or the South East Health Care Regions of Sweden (1987–99) following a TURP for symptomatic benign prostatic hyperplasia [13]. All study participants were placed under surveillance for disease progression, but did not undergo initial treatment following the standard protocols at that time. We excluded five cases missing the date of tumor tissue collection, leaving a total of 362 cases. The gene

profiling study design was approved by the Ethical Review Boards in Örebro and Linköping [14].

The Physicians' Health Study I and II are two randomized clinical trials of aspirin and micronutrients for the primary prevention of cardiovascular disease and cancer. The trial began in 1982 and eventually included a total of 29,067 men aged 40–84 in either trial [15, 16]. We included in the gene expression profiling study tumor tissue samples collected from 116 cases in the Physicians' Health Study (PHS) diagnosed 1983–2003 and treated with RP as their primary treatment or who were diagnosed following a TURP [13]. We excluded seven participants with low quality expression data and three cases missing the date of tumor tissue collection from the analysis, leaving a total of 106 cases. The gene expression profiling study was approved by the Human Subjects Committee at Brigham and Women's Hospital, and the Institutional Review Board at Harvard School of Public Health [13].

We also had data available on VDR protein tumor expression for 316 cases from the PHS (diagnosed 1983–2004), and 525 cases from the Health Professionals Follow-Up Study (diagnosed 1986–2002), a prospective cohort of 51,529 US male health professionals enrolled in 1986 [17]. Cases in the Health Professionals Follow-Up Study (HPFS) included in this analysis were treated with radical prostatectomy [12]. Lethal cases were defined as those who developed distant metastases or who died from prostate cancer during follow-up through 2010. Nonlethal cases were defined as those who were diagnosed with stage T1 or T2 prostate cancer with no evidence of metastasis, and who did not die from prostate cancer. Cases from the PHS (n=5) and HPFS (n=4) were excluded from this analysis if missing complete and valid information regarding date of tissue collection or date of diagnosis.

Gene expression profiling

Detailed methods for the design, implementation, and quality assessment of gene expression profiling have been published for both the PHS and Swedish cohorts [13, 14]. RNA was quantified using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE) [13]. As reported in previous publications, RNA quality was assessed by the SYBR green (QIAGEN Inc, Valencia, CA) quantitative polymerase chain reaction assay for housekeeping gene RPL13A, with a crossover threshold <30 considered acceptable for inclusion in the DASL assay [13]. Gene expression profiling was conducted on four complementary DNA (cDNA)-mediated annealing, selection, ligation and extension (DASL) assay panels containing over 6100 genes (6K DASL) [18, 19]. Details on the creation of the array can be found at Gene Expression Omnibus with platform accession number GPL5474 (<http://www.ncbi.nlm.nih.gov/geo/>). From both the Swedish and PHS cohorts, high quality expression data were obtained from 93.65% of the original prostate cancer samples [13]. The array included mRNA expression of six genes involved in the vitamin D metabolic pathway: group-specific component (GC), vitamin D receptor (VDR), three members of the cytochrome P450 family (CYP27A1, CYP27B1, CYP24A1), and retinoid X receptor-alpha (RXR α).

VDR Protein Expression

Immunohistochemistry of VDR protein expression was previously undertaken on nine tumor tissue microarrays from 841 men with prostate cancer from the PHS and HPFS using a VDR antibody (anti-VDR rabbit polyclonal [C20], sc-1008; Santa Cruz Biotechnology, Santa Cruz, CA). VDR staining intensity was analyzed using the CRi Vectra semi-automated quantitative image analysis system (CRi, Woburn, MA) [12]. VDR expression was measured as a combination of the percentage of area per core that is positively stained and the intensity of staining; final scores were averaged across cores for each participant. In an earlier publication using this data, inpatient variability of VDR expression was examined

by calculating intraclass correlation coefficients across a patient's TMA cores; high concordance was found, with an ICC of 77.2 [12].

Statistical analysis

We conducted separate analyses of mRNA gene expression by study population (PHS and Swedish cohorts) due to differences in tissue origin, selection criteria, and potential for UV exposure from sunlight. Analysis of VDR protein expression was conducted by study population as well as in the combined cohorts of PHS and HPFS participants. Seasons were defined according to calendar month of tissue collection: winter (December, January, February), spring (March, April, May), summer (June, July, August, September), and autumn (October, November). September was included as a summer month to account for increased vitamin D expression resulting from heightened sun exposure during the summer season.

One-way analysis of variance (ANOVA) was used to compare mean expression levels of each gene, as well as VDR protein, across seasons. Gene expression levels were log-transformed to improve normality. Models were adjusted for age at diagnosis (continuous) and Gleason grade (Gleason score 2–6; 7; or 8–10); models for VDR protein expression were additionally adjusted for study (PHS, HPFS). Variation was also examined separately among lethal and nonlethal cases, adjusted for age at diagnosis, to determine if the association varied by disease severity.

In addition, we assessed variation in gene expression by seasonal exposure to ultraviolet radiation, assigning mean monthly ultraviolet radiation levels based on the US Environmental Protection Agency monthly average UV Index (for the PHS and HPFS) [20] and the Swedish Radiation Safety Authority (SSM) 2010 Report on ultraviolet radiation (for the Swedish cohort) [21]. US values were based on latitude 40°N, and Swedish values were based on latitude 60°N, approximately central locations for both countries. Mean monthly UV levels were assessed as a semi-continuous variable, and included in a linear regression model with gene expression levels as the dependent variable. Beta coefficients from the linear regression model represented the change in gene expression levels per unit increase in the UV Index. Models for the semi-continuous assessment of mean monthly UV exposure were adjusted for age at diagnosis and Gleason grade. Models of VDR protein expression with the PHS and HPFS cohorts combined were additionally adjusted for study. Analyses were conducted using SAS v9.2 (SAS Institute, Cary, NC).

Results

Our analysis of gene expression levels (mRNA) included 362 Swedish cases from the Watchful Waiting cohort (170 lethal, 192 indolent), and 106 US cases from the PHS cohort (27 lethal, 79 indolent) with expression data on all six studied genes and complete dates of tissue collection. Swedish cases were on average older than PHS cases at diagnosis (73.9 vs. 67.3 years). Our analysis of VDR protein expression included 311 cases from the PHS, and 521 cases from the HPFS cohort with complete dates of tissue collection. Mean age at diagnosis did not vary substantially by season of tissue extraction in any of the study populations. There was no consistent pattern in the proportion of cases with high Gleason grade tumors by season across populations (Table 1).

We found no statistically significant variation in mRNA expression of the six genes in the vitamin D pathway by season of tissue collection in either the Swedish or PHS cohort (Table 2). However, there was suggestive seasonal variation in mRNA expression of VDR among lethal cases in the PHS ($p=0.02$), with lowest expression levels in the winter, and highest levels in the summer. This finding was not replicated among lethal cases in the Swedish

cohort. Among all cases, there was no significant change in mRNA expression of the six genes per unit increase in UV radiation when season was assessed as a semi-continuous variable based on average monthly UV radiation exposure (p-values 0.14, Supplemental Table 1). However, among lethal cases in the PHS, VDR gene expression varied by monthly UV radiation exposure (p=0.003), with slightly higher levels of VDR expression with increasing UV radiation. There was also variation in CYP24A1 expression among indolent cases in the Swedish cohort (p=0.04), with slightly lower expression levels with increasing UV radiation; however, the findings were not replicated between studies.

We found no statistically significant seasonal variation in VDR protein expression when season was assessed categorically among all cases (Table 3). However, despite small numbers, variation was significant among lethal cases in the PHS (p=0.02), with a 6-fold higher VDR expression in summer (n=3 lethal cases) compared to winter (n=6), and a more than 5-fold higher level in fall (n=6) compared to winter (n=6). VDR protein expression did not display seasonal variation among nonlethal cases (p=0.39). We did not replicate this finding in lethal and nonlethal cases in the HPFS, or when the cohorts were combined. As previously reported, lethal cases had significantly lower mean VDR protein expression levels than nonlethal cases in the combined cohorts (p=0.0002) [12]. When season was assessed as a semi-continuous variable based on average monthly UV radiation exposure using linear regression in the combined cohorts, we found suggestive variation in VDR protein expression among all cases (p=0.06) and among nonlethal cases (p=0.01). VDR protein levels increased 50% and 90% per unit increase in UV index among all cases and nonlethal cases, respectively. There was no association among lethal cases (p=0.41).

Discussion

Unlike the seasonal variation in circulating levels of 25(OH)D, we found no overall statistically significant variation in mRNA expression of six vitamin D genes or VDR protein levels by season of tissue collection when assessed categorically. As a result, it is unlikely that epidemiological analyses of these biomarkers of the vitamin D pathway expressed in prostate tissue are biased by season of tissue collection. However, mRNA expression of VDR varied among lethal cases in the PHS when season was assessed categorically and as a semi-continuous variable representative of average monthly UV exposure. VDR protein expression also varied seasonally among lethal cases in the PHS when season was assessed categorically; however, these results were not replicated in the other study populations, and the number of lethal cases was small. We also observed significant variation in VDR protein expression among all cases and nonlethal cases in the combined HPFS and PHS cohorts with season assessed as average monthly UV exposure. VDR binds 1,25(OH)₂D₃, which then forms a heterodimer complex with RXR to bind vitamin D responsive elements [22]. It is possible that disease severity affects the expression of genes in prostate tissue, though the literature is sparse on this issue. The inconsistent observations of seasonal variation in VDR gene and protein expression among subgroups of prostate cancer cases may be a chance finding; this observation requires confirmation in other populations.

This study has potential limitations to consider. We assessed season by month of tissue collection as our best estimate of seasonal UV exposure. We attempted to account for differences in exposure to UV radiation between study populations by modeling the mean monthly UV levels for each study population. This is an imprecise measure and many other components, including time spent outdoors and exact latitude, contribute to environmental UV exposure. However, prior studies of circulating vitamin D have demonstrated an association between serum levels and season. In addition, we did not incorporate dietary or

supplemental vitamin D intake into our analysis, which may contribute to gene expression levels particularly in the winter when dermal synthesis of vitamin D is low.

The number of lethal cases included in this analysis was small, and a larger study may increase the precision of the estimates and overall power to detect seasonal variation in gene expression. We cannot rule out chance as an explanation for our findings. Furthermore, our tissue samples came from two sources, TURP and RP. TURP tends to target the transitional zone of the prostate, while tumors present in a RP sample often occur in the peripheral zone. It is possible that vitamin D expression levels differ by prostate zone, but we were unable to assess this in further detail.

Fewer prostate cancer cases are diagnosed in the summer in Sweden due to a number of factors, including traditional vacation time among Swedish citizens, and resulting limited access to healthcare during these months. As such, men presenting with prostate cancer in the summer may have more severe symptoms, and perhaps more aggressive disease than men presenting at other times of the year [23]. This could affect our results, as gene expression levels measured during the summer could be confounded by disease severity. We attempted to address this issue by adjusting for Gleason grade at diagnosis, a strong predictor of survival [24].

Our analysis has several strengths, including the investigation of our hypothesis in multiple study populations. We were also able to adjust models for Gleason grade at diagnosis, and explore the association by lethal or nonlethal outcome status. We assessed the expression of six genes at various points along the vitamin D metabolic pathway. In addition, we assessed VDR at both mRNA and protein levels to examine different aspects of expression.

In conclusion, we found no overall variation in expression levels of genes on the vitamin D pathway by season of tissue collection. There was a suggestive association between mean monthly UV levels and VDR gene and protein expression, but results were inconsistent between population subgroups. Future analyses of the association between tissue biomarkers of the vitamin D pathway and prostate cancer are unlikely to be biased by seasonality; however our results should be confirmed in other populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of the study populations by season of TURP/RP^a

	Gene expression analysis						VDR protein analysis					
	Swedish Watchful Waiting cohort (n=362)			Physicians' Health Study (n=106)			PHS and HPFS combined cohorts ^b (n=832)					
	Spring	Summer	Autumn	Winter	Spring ^c	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Total number of cases (%)	99 (27%) ^d	57 (16%)	86 (24%)	120 (33%)	37 (35%)	24 (23%)	21 (20%)	24 (23%)	204 (25%)	277 (33%)	142 (17%)	209 (25%)
Age at diagnosis, Mean (SD)	73.9 (7.1)	73.4 (6.9)	72.7 (7.4)	73.5 (7.0)	66.3 (7.5)	69.1 (7.1)	67.3 (6.8)	67.1 (6.1)	65.6 (6.2)	65.9 (6.5)	65.7 (6.5)	65.7 (6.1)
Gleason grade, n (%)												
Low (2–6)	30 (28%)	17 (16%)	29 (27%)	33 (30%)	5 (42%)	2 (17%)	2 (17%)	3 (25%)	44 (24%)	58 (32%)	28 (15%)	54 (29%)
Medium (7)	43 (27%)	26 (16%)	37 (23%)	56 (35%)	23 (36%)	15 (23%)	11 (17%)	15 (23%)	129 (25%)	178 (35%)	80 (16%)	119 (24%)
High (8–10)	26 (29%)	14 (15%)	20 (22%)	31 (34%)	9 (30%)	7 (23%)	8 (27%)	6 (20%)	31 (22%)	41 (29%)	34 (24%)	36 (25%)
Lethal Prostate Cancer ^e , n (%)	39 (23%)	30 (18%)	52 (31%)	49 (29%)	11 (41%)	5 (19%)	5 (19%)	6 (22%)	21 (28%)	21 (28%)	13 (17%)	21 (28%)
Nonlethal Prostate Cancer ^f	60 (31%)	27 (14%)	34 (18%)	71 (37%)	26 (33%)	19 (24%)	16 (20%)	18 (23%)	146 (24%)	197 (33%)	101 (17%)	157 (26%)

^aTURP: Trans-urethral resection of the prostate; RP: Radical Prostatectomy

^bVDR protein analysis includes prostate cancer cases from the Physicians' Health Study (n=311) and Health Professionals Follow-up Study (n=521) cohorts

^cSpring: March, April, May; Summer: June, July, August, September; Autumn: October, November; Winter: December, January, February

^dPercentages may not add to 100 due to rounding error

^eCases diagnosed with distant metastases or who died from prostate cancer

^fNonlethal disease includes long-term survivors (10 yrs) in gene expression analyses, and cases diagnosed at stage T1 or T2 and M0 who did not die of prostate cancer

Table 2

Mean seasonal expression levels of vitamin D pathway genes overall and stratified by lethal or indolent disease status

Swedish Watchful Waiting Cohort (n=362)						Physicians' Health Study (n=106)				
	Spring ^a	Summer	Fall	Winter	p-values ^b	Spring	Summer	Fall	Winter	p-values
GC										
All cases	9.12 (0.73) ^e	9.04 (0.73)	9.17 (0.76)	9.04 (0.67)	0.49	8.77 (0.52)	9.30 (1.21)	8.78 (0.52)	9.12 (0.93)	0.08
Lethal ^c	9.14 (0.86)	9.17 (0.86)	9.25 (0.81)	9.00 (0.69)	0.44	8.79 (0.61)	9.22 (1.15)	8.38 (0.09)	9.56 (1.46)	0.18
Nonlethal ^d	9.10 (0.63)	8.89 (0.53)	9.06 (0.68)	9.07 (0.66)	0.51	8.77 (0.49)	9.32 (1.26)	8.91 (0.53)	8.97 (0.68)	0.19
VDR										
All cases	8.67 (0.58)	8.58 (0.44)	8.60 (0.59)	8.65 (0.67)	0.74	8.29 (0.42)	8.34 (0.46)	8.24 (0.39)	8.33 (0.41)	0.92
Lethal	8.70 (0.57)	8.56 (0.47)	8.59 (0.59)	8.63 (0.54)	0.74	8.27 (0.28)	8.77 (0.40)	8.32 (0.56)	8.11 (0.31)	0.02
Nonlethal	8.66 (0.59)	8.60 (0.42)	8.62 (0.61)	8.67 (0.76)	0.95	8.39 (0.43)	8.23 (0.41)	8.22 (0.34)	8.39 (0.43)	0.55
CYP24A1										
All cases	12.11 (1.26)	12.06 (1.11)	12.34 (1.08)	12.10 (1.20)	0.46	11.52 (1.14)	11.34 (1.13)	11.74 (0.99)	11.92 (1.08)	0.27
Lethal	12.30 (1.12)	12.16 (0.97)	12.23 (1.17)	12.01 (1.14)	0.64	11.39 (1.13)	11.08 (1.25)	11.27 (1.04)	11.89 (0.96)	0.79
Nonlethal	11.99 (1.34)	11.94 (1.27)	12.51 (0.91)	12.17 (1.24)	0.19	11.58 (1.16)	11.41 (1.13)	11.88 (0.96)	11.92 (1.14)	0.42
CYP27A1										
All cases	11.97 (0.94)	11.96 (0.89)	11.93 (0.90)	11.95 (0.90)	0.98	11.24 (0.82)	11.31 (0.84)	11.28 (0.87)	11.35 (0.70)	0.89
Lethal	11.90 (0.99)	11.87 (0.74)	11.74 (0.92)	11.88 (0.90)	0.90	10.84 (0.55)	11.14 (1.24)	10.73 (0.68)	10.96 (1.09)	0.91
Nonlethal	12.02 (0.91)	12.06 (1.03)	12.24 (0.81)	12.00 (0.91)	0.72	11.41 (0.87)	11.35 (0.74)	11.45 (0.87)	11.48 (0.49)	0.96
CYP27B1										
All cases	9.45 (0.88)	9.47 (0.85)	9.45 (0.79)	9.62 (0.78)	0.29	9.26 (0.80)	9.29 (0.86)	9.29 (0.71)	9.28 (0.79)	0.97
Lethal	9.36 (0.83)	9.55 (0.77)	9.42 (0.81)	9.62 (0.75)	0.40	8.78 (0.63)	9.24 (1.13)	8.91 (0.61)	9.19 (0.76)	0.64
Nonlethal	9.50 (0.91)	9.38 (0.93)	9.50 (0.77)	9.62 (0.80)	0.59	9.47 (0.79)	9.30 (0.81)	9.40 (0.72)	9.32 (0.81)	0.96
RXRα										
All cases	12.79 (0.41)	12.89 (0.24)	12.77 (0.40)	12.81 (0.35)	0.28	12.81 (0.44)	12.84 (0.34)	12.72 (0.27)	12.83 (0.36)	0.65
Lethal	12.87 (0.42)	12.89 (0.24)	12.70 (0.48)	12.82 (0.31)	0.07	12.99 (0.25)	12.94 (0.47)	12.58 (0.40)	12.88 (0.33)	0.18
Nonlethal	12.74 (0.40)	12.88 (0.25)	12.87 (0.22)	12.80 (0.38)	0.22	12.74 (0.48)	12.81 (0.30)	12.76 (0.22)	12.81 (0.37)	0.91

^aSeasons defined as Spring: March, April, May; Summer: June, July, August, September; Fall: October, November; Winter: December, January, February

^bP-values from one-way ANOVA; overall prostate cancer adjusted for age and Gleason grade at diagnosis (2–6, 7, 8–10), lethal and indolent adjusted for age

^c Lethal disease defined as all cases who died from prostate cancer or those diagnosed with distant metastases; Sweden n=170; PHS n=27

^d Nonlethal disease defined as all cases who survived 10 years after diagnosis; Sweden n=192; PHS n=79

^e Mean (SD) for all such values on original scale

Table 3

Variation in VDR protein expression by season of tissue extraction, as assessed by season of tissue extraction and mean monthly ultraviolet (UV) radiation exposure

	N	Mean expression levels				Expression by mean monthly UV radiation exposure		
		Spring ^a	Summer	Fall	Winter	p-value	Beta ^b	p-value ^c
PHS								
All cases ^d	311	29.04	33.30	34.61	27.57	0.11	0.46	0.21
Lethal ^e	21	13.99	38.38	31.85	5.97	0.02	3.16	0.04
Nonlethal ^f	290	30.17	33.25	34.81	29.47	0.39	0.25	0.51
HPFS								
All cases	521 ³	32.64	30.47	27.39	29.31	0.28	0.37	0.18
Lethal	55	27.22	21.43	17.25	27.90	0.43	−0.10	0.89
Nonlethal ^g	327	33.16	35.21	29.86	29.19	0.14	0.88	0.01
Combined ^h								
All cases	832	31.30	31.49	30.27	28.68	0.43	0.41	0.06
Lethal	76	23.98	22.60	24.68	21.93	0.97	0.56	0.41
Nonlethal	601	32.38	34.52	32.21	29.33	0.12	0.65	0.01

^aSeasons defined as Spring: March, April, May; Summer: June, July, August, September; Fall: October, November; Winter: December, January, February

^bBeta coefficients from linear regression models, adjusted for age at diagnosis (all models) and Gleason grade (overall only), represent a one-unit increase in UV Index

^cP-values from UV index variable in linear regression model

^dP-values for models of all cases from one-way ANOVA adjusted for age and Gleason grade at diagnosis

^eLethal cases were diagnosed with distant metastasis or died from prostate cancer; P-values from one-way ANOVA adjusted for age at diagnosis

^fNonlethal cases survived 10 years after diagnosis; P-value from one-way ANOVA adjusted for age at diagnosis

^gNonlethal cases diagnosed at stage T1-T2/M0 and did not die from prostate cancer; P-value from one-way ANOVA adjusted for age at diagnosis

^hModels additionally adjusted for study