# Associations of Circulating 25-hydroxyvitamin D with prostate cancer diagnosis, stage and grade

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## Abstract

Epidemiological studies suggest that vitamin D protects against prostate cancer, although evidence is limited and inconsistent. We investigated associations of circulating total 25-hydroxyvitamin D (25(OH)D) with PSA-detected prostate cancer in a case-control study nested within the Prostate Testing for Cancer and Treatment (ProtecT) trial. Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) quantifying the association between circulating total 25(OH)D and prostate cancer. In case-only analyses, we used unconditional logistic regression to quantify associations of total 25(OH)D with stage (advanced vs localized) and Gleason grade (high-grade ( 7) vs low-grade (<7)). Pre-determined categories of total 25(OH)D were defined as: high:  30ng/mL; adequate: 20-<30ng/mL; insufficient: 12-<20ng/mL; deficient: <12ng/mL. Fractional polynomials were used to investigate the existence of any U-shaped relationship. We included 1,447 prostate cancer cases (153 advanced, 469 high-grade) and 1,449 healthy controls. There was evidence that men deficient in vitamin D had a two-fold increased risk of advanced versus localized cancer (OR for deficient vs adequate total 25(OH)D=2.33, 95% CI: 1.26,4.28) and high-grade versus low-grade cancer (OR for deficient vs adequate total 25(OH)D=1.78, 95% CI: 1.15,2.77). There was no evidence of a linear association between total 25(OH)D and prostate cancer (p=0.44) or of an increased risk of prostate cancer with high and low vitamin D levels. Our study provides evidence that lower 25(OH)D concentrations were associated with more aggressive cancers (advanced versus localized cancers and high- versus low- Gleason grade), but there was no evidence of an association with overall prostate cancer risk.

### Keywords

Prostate cancer; vitamin D; 25-hydroxyvitamn D

## Introduction

Prostate cancer is the most common male cancer in industrialized countries but knowledge of modifiable risk factors is limited. Ecological studies have reported lower prostate cancer

mortality at latitudes closer to the equator[1], leading to speculation that 25-hydroxyvitamin D (25(OH)D), which is mainly obtained from sunlight exposure, may protect against prostate cancer[2]. Metabolites of vitamin D control cellular growth and differentiation[3-4] and administration of vitamin D analogues inhibits the progression of prostate cancer in animal models[5-6] and in phase II trials[7].

In line with ecological studies and understanding of the biological actions of vitamin D, epidemiological studies have shown inverse associations of circulating total 25(OH)D with prostate cancer risk at the individual level[3, 8-9]. Overall, however, the evidence is inconsistent, with our recent meta-analyses finding little evidence that either increased life-course sun exposure, dietary vitamin D or circulating concentrations of total 25(OH)D or 1,25-dihydroxyvitamin D (1,25(OH)$_2$D (the active form of the hormone) are associated with prostate cancer[10-12]. There has been some support for a protective role of 25(OH)D in advanced prostate cancers[5-6, 13], although this is limited by few studies and small sample sizes (n=62 to 460) with some studies reporting an increased risk of advanced prostate cancer with increased total 25(OH)D[10, 14-15] or a possible U-shaped association[16]. Few studies include exclusively PSA-detected prostate cancer, of increasing relevance in the PSA-era due to earlier detection of localized disease.

We investigated associations of circulating total 25(OH)D with PSA-detected prostate cancer, overall and stratified by stage and grade in a large UK-wide population-based case-control study[17]. The sample size (n=1447 cases) is twice the size of the largest previously published study of total 25(OH)D in prostate cancer (n=749). We hypothesized that lower levels of total 25(OH)D are associated with an increased risk of prostate cancer, and that the association is stronger for advanced versus localized, and high-grade versus low-grade, cancer. We also investigated the possibility of a U-shaped relationship (i.e. whether both high and low levels of total 25(OH)D are associated with an increased risk of prostate cancer).

## Materials and Methods

### Participants

The study is nested within the prostate cancer detection phase for a multi-centre randomized controlled trial of treatments for localized disease: the Prostate Testing for cancer and Treatment (ProtecT) study (ISRCTN20141297)[17]. During recruitment to the ProtecT study (between 2001 and 2009), over 100,000 men aged 50-69 years at 337 general practices in nine UK centres (Birmingham, Bristol, Cambridge, Cardiff, Edinburgh, Leeds, Leicester, Newcastle, Sheffield) were offered a PSA test at a community-based 'prostate check clinic', and those with raised levels ( 3 ng/ml) were offered diagnostic biopsy. Detected tumours were all histology-confirmed and clinically staged using the TNM system[18]. Cancer stages T1-T2 and NX,M0 were categorised as 'localized'; and T3-T4, N1,M1 as 'advanced'. 'High grade' tumours were defined as a Gleason score 7 and low-grade as Gleason score <7, after review of biopsy cores by a specialist uro-pathologist.

### Case Control Selection

A total of 1,914 cases and 48,692 controls were potentially eligible for selection for the current study (based on men recruited between 2003 and 2008), had provided a plasma heparin sample and consented to prostate cancer research. We randomly selected 1500 cases and one stratum-matched control for each case from those men who had provided a non-fasted blood sample at the prostate check clinic. Controls were randomly selected from the same stratum - i.e. five-year age-band (age at PSA test) and GP/family practice - as cases. Prostate check clinics were held over consecutive weeks at each GP practice, and so

matching cases and controls by GP also matches on time and season of blood draw. All participants in the ProtecT prostate check clinics who had no evidence of prostate cancer were eligible for selection as controls; that is, men with a PSA test <3ng/mL or a raised PSA ( 3ng/mL) combined with at least one negative biopsy and no subsequent prostate cancer diagnosis during the follow-up protocol for negative biopsies. All men provided written informed consent prior to inclusion in the study. Trent Multicentre Research Ethics Committee (MREC) approved the ProtecT study (MREC/01/4/025) and the associated ProMPT study which collected biological material (MREC/01/4/061).

## Vitamin D Assay

Plasma samples drawn into heparinised tubes at the prostate check clinic were allowed to clot at room temperature, and then were centrifuged for 20 minutes within two hours of collection at 1640 relative centrifugal force. Samples were stored at −80°C, until required for use. For each man, samples were retrieved from storage, thawed and processed by a LabMan™ automated blood reformatting robot, and 500µl of plasma extracted, frozen within 36 hours and sent for 25(OH)D analysis.

The samples were assayed in 31 batches over a period of approximately three months. $25(OH)D_2$ and $25(OH)D_3$ were measured in plasma which was deproteinised using zinc sulphate and acetonitrile. A deuterated internal standard was incorporated within every sample analysis (hexadeuterium-25-hydroxyvitamin $D_3$, Synthetica AS, Norway). Samples were measured on a Tandem MS system utilizing chromatographic separation on a Waters Sunfire C18 column (Waters, Manchester UK) using methanol, ammonium acetate and formic acid mobile phase. Detection of $25(OH)D_2$ and $25(OH)D_3$ was achieved using a Micromass Quatro Ultima Pt fitted with a Z spray ESI inlet. Excellent chromatographic resolution of the peaks of interest allows use of the primary daughter ions from water loss transitions. The assay was standardized using NIST aligned standard material obtained from Chromsystems (UK). Between batch coefficients of variation for $25(OH)D_2$ were 4.2% - 5.5%, and for $25(OH)D_3$ were 4.5%-5.7%, across the assay working range.

Circulating concentrations of $25(OH)D_2$ and $25(OH)D_3$ were measured in nanograms per millilitre (ng/mL) where 1ng/mL=2.5nmol/L (nanomoles per litre). Total 25(OH)D (ng/mL) was calculated as the summation of $25(OH)D_2$ and $25(OH)D_3$.

## Other Covariates

Measures of height, weight, weekly exercise, smoking status, family history of prostate cancer, history of benign prostatic hyperplasia (BPH), diabetes, systolic and diastolic blood pressure, occupational social class, and self-reported ethnicity were collected at the time of the initial PSA test, either by questionnaire or by nurse interview[19]. These measures were obtained prior to knowledge of the PSA level or diagnosis in 85% of men. We calculated body mass index (BMI; $kg/m^2$), which represents general adiposity. We calculated mean arterial pressure (MAP=(2*diastolic + systolic)/3; mmHg), which represents average arterial blood pressure during a single cardiac cycle.

## Statistical Analysis

Mean (and 95% confidence interval (CI)) concentrations of age-adjusted total 25(OH)D were calculated for quantiles of age-group (50-54, 55-59, 60-64, 65), study centre location (North: Sheffield, Newcastle, Edinburgh, Leeds; South: Bristol, Cardiff, Birmingham, Leicester, Cambridge ) and season of blood draw (Winter: January, February, March; Spring: April, May, June; Summer: July, August, September; Autumn: October, November, December). Seasonal variation in mean total 25(OH)D per calendar week of blood collection was estimated using locally weighted polynomial regression[20] which smoothes long term

trends. To allow for the matched sets of cases and controls, conditional logistic regression, was used to estimate odds ratios (OR) and 95% confidence intervals (CI) quantifying the association between circulating concentrations of total 25(OH)D and all prostate cancers. The model included all case-control matching variables (5-year age band and GP surgery), as well as assay batch and exact age. Matching on GP surgery accounted for study centre (i.e. geographical location) and season of blood draw, as clinics were held over a number of consecutive weeks at each GP surgery. A case-only analysis used unconditional logistic regression, controlling for assay batch, age, GP surgery, study centre and season of blood draw, to quantify associations of circulating concentrations of total 25 (OH)D with more aggressive prostate cancer i.e. prostate cancer stage (advanced vs localized) and Gleason grade (high-grade ( 7) vs low-grade (<7)). A case-only analysis was used as all cases have undergone biopsy, therefore removing potential detection bias which could occur through misclassification of control status because of imperfect sensitivity of the PSA test. An odds ratio of greater than 1 indicates that the exposure (low total 25(OH)D) is more strongly associated with advanced cancer compared to localized cancer, or with high-grade cancer compared with low-grade cancer. The vitamin D exposure was based on pre-determined categories of total 25(OH)D , defined as: high:  30ng/mL; adequate: 20-<30ng/mL; insufficient: 12-<20ng/mL; deficient: <12ng/mL We also computed ORs and 95% CIs for associations of prostate cancer per standard deviation decrease in 25(OH)D.

Sensitivity analyses were carried out additionally adjusting for family history of prostate cancer, BMI, weekly exercise, diabetes and smoking status. However these variables are not thought to be strongly related to prostate cancer risk and sensitivity analyses confirmed that they did not confound observed associations.

Sensitivity analyses were carried out to assess the impact of additionally adjusting for sample storage time and using a square-root transformation of total 25(OH)D. Comparisons of fractional polynomials[21] with between one and two degrees were used to investigate the existence of a U-shaped relationship between continuous total 25(OH)D and prostate cancer.

It has been suggested that complete-case analyses, based only on those men with information on all confounding variables, may produce biased effect estimates[22-23]. To avoid this we included all men in the analysis by multiply imputing missing covariate values (i=10) using chained equations[24], assuming those values could be predicted without bias from the observed relationships between covariates and the outcome measure, and substituting imputed values for missing values. A sensitivity analysis, using complete cases only, determined whether the potential for biased estimates due to missing data was realised.

Analyses were carried out in Stata 11 (StataCorp, 2009. College Station, TX) using the -ice- for multiple imputation with chained equations[24] for imputing missing data. All tests of statistical significance were two-sided.

## Results

### Baseline Characteristics of Study Participants

There were a total of 2,992 eligible men whose blood samples were sent to the laboratory (1,496 cases, 1,496 controls). Three were wrongly labelled so that the results could not be matched back to the patient's details and were discarded (2 cases, 1 control). There were 93 men (47 cases, 46 controls) for whom results were unusable due to insufficient sample. We therefore included 1,447 prostate cancer cases and 1,449 controls that had a total 25(OH)D measurement available. Of the 1,447 cases, 1,289 (89.1%) were localized cancers, 153 (10.6%) were advanced cancers and 5 (0.3%) were not staged. There were 974 (67.3%) low-

grade cases (Gleason grade <7), 469 (32.4%) high-grade cases (Gleason grade 7) and 4 (0.2%) cases with no grade.

The baseline characteristics of cases and controls are shown in Table 1. Of the 99.3% of subjects who had recorded ethnicity, 98.9% self-identified as white. The mean age in subjects was 62.5 years (range: 50.3-71.4). Men with localized/low-grade cancer were slightly younger than men with advanced/high-grade cancer. There were no substantial differences between cases and controls, except that more cases had a family history of prostate cancer versus controls and fewer cases had diabetes. Mean arterial pressure was slightly higher in cases than controls. Men with advanced/high-grade cancer had a higher age-adjusted PSA than men with localized/low-grade cancer (data not shown). There was no meaningful difference between the mean storage times of blood samples between cases and controls.

### Characteristics of cases and controls by 25(OH)D Concentration

In our 2,896 men, the overall unadjusted mean total 25(OH)D was 23.0ng/mL (inter-quartile range (IQR): 17.1, 28.0). There was no correlation between PSA and total 25(OH)D level ($\rho$=−0.03). There were no differences in total 25(OH)D between cases and controls by age-group, study centre location or season of blood draw (Table 2). As expected, men whose blood was drawn in winter had lower total 25(OH)D (Table 2 and Figure 1), but unexpected was that men from study centres in the south of the UK had lower total 25(OH)D (p=0.005).

Mean total 25(OH)D did not differ between all cases and controls (23.0ng/mL vs 22.9ng/mL respectively, p for difference= 0.72). However, mean total 25(OH)D was lower in men with advanced stage cancers than localized cancers (21.6 (7.9) ng/mL vs 23.2 (8.8) ng/mL, p for difference=0.03), and also in men with high-grade cancers versus low-grade cancers (22.4 (8.5) ng/mL vs 23.3 (8.8) ng/mL, p for difference=0.05).

### Concentrations of Total 25(OH)D and Prostate Cancer Risk

In conditional logistic regression models, there was no evidence of an association between circulating total 25(OH)D concentration and case control status (p=0.83) (Table 3). Comparing advanced/high-grade cases with controls, but not localized/low-grade cases with controls, using conditional logistic regression models showed weak evidence of an inverse association (Table 4). However, there was stronger evidence of an association between lower total 25(OH)D and higher risk of advanced versus localized prostate cancer in case-only analyses (Table 4), which remained after adjusting for confounding variables (OR for deficient vs adequate total 25(OH)D=2.33, 95% CI: 1.26,4.28; p for difference=0.01). There was evidence of a linear trend, with increasing risk of advanced prostate cancer as total 25(OH)D levels decreased from high to deficient (OR for advanced cancer per standard deviation decrease in total 25(OH)D = 1.30, 95% CI: 1.06,1.59; p linear trend = 0.01). Similarly, there was evidence of an association between decreasing total 25(OH)D and risk of high-grade versus low-grade prostate cancer (OR of high-grade cancer for deficient vs adequate total 25(OH)D=1.78, 95% CI: 1.15,2.77; p for difference=0.01) (Table 4). The OR of high-grade cancer per standard deviation decrease in total 25(OH)D was 1.14, 95% CI: 1.00,1.29 (p=0.04). Additionally adjusting for family history, BMI, diabetes, smoking status, weekly exercise or storage time of the sample did not alter the results. Complete case analysis produced equivalent odds ratios to using the dataset with imputed missing co-variates. Results did not change if per standard deviation of 25(OH)D$_3$ was included instead of total 25(OH)D. Fractional polynomials found no evidence of a U-shaped relationship between total 25(OH)D and prostate cancer, and there did not appear to be a U-shaped relationship for advanced prostate cancer.

# Discussion

## Summary of Findings

This study, of 1,447 prostate cancer cases and 1,449 healthy controls from the ProtecT study, investigated associations of circulating plasma total 25(OH)D with prostate cancer risk and, in cases, with stage and Gleason grade. There was evidence of a two-fold increased risk of advanced versus localized prostate cancer and high-grade versus low-grade cancer in men deficient in vitamin D. There was no evidence of an association between total 25(OH)D and overall prostate cancer incidence (p=0.50).

Our findings agree with some previous epidemiological studies. Our published systematic review and meta-analysis found no overall association between total 25(OH)D concentrations and prostate cancer (14 studies, 4353 prostate cancer cases; random-effects OR per 10ng/mL increase in total 25(OH)D=1.04, 95% CI: 0.99,1.10; p=0.12), or aggressive prostate cancer (6 studies, 871 aggressive prostate cancer cases; random-effects OR per 10ng/mL increase in total 25(OH)D=0.98, 95% CI:0.84,1.15; p=0.78)[12]. There was, however, weak evidence of a possible small decreased risk of aggressive prostate cancer with increasing 1,25(OH)2D (2 studies, 696 aggressive prostate cancer cases; random-effects OR per 10pg/mL increase in 1,25(OH)2D =0.86, 95% 0.72,1.02; p = 0.09). This result was based on only two studies, so confidence intervals were wide, but a potentially important protective effect of 1,25(OH)2D on advanced prostate cancer could not be excluded. 'Aggressive' prostate cancer was defined as advanced stage and/or high grade (as defined in individual papers) and the analysis compared cases with controls, rather than case-only analyses. Our current analysis supports others in indicating no association between total 25(OH)D and overall prostate cancer risk, but does support previous epidemiological and in vivo studies, suggesting that higher levels of vitamin D protect against more aggressive forms of prostate cancer amongst cases. A recent paper by Fang et al[25] also found evidence that prostate cancer patients with lower 25(OH)D had a higher risk of developing metastatic or fatal prostate cancer.

The association between total 25 (OH)D and more aggressive prostate cancers amongst cases is consistent with experimental data showing that low total 25 (OH)D concentrations reduce differentiation, and enhance cancer invasion and angiogenesis[3-4, 6]. Prostate cancer cells have reduced 1-alpha-hydroxylase activity as compared to normal prostate epithelial cells, and thus have a reduced ability to locally convert 25(OH)D to $1,25(OH)_2D$[26]. Genes related to the vitamin D pathway suggest a link with advanced disease in particular, with a recent genetic association study and meta-analysis of 13 studies finding an association between three vitamin D receptor gene (VDR) polymorphisms (BsmI, ApaI, and TaqI) and prostate cancer grade[27]: ApaI-a and BsmIb increased the risk of a high grade, TaqI-t was protective. These polymorphisms modulate the activity of the VDR and therefore may modulate a protective effect of $1,25(OH)_2D$ on aggressive prostate cancers.

Some studies show positive associations of both low and high total 25(OH)D[14, 28-30] with prostate cancer, but a review by Yin et al[16] concluded that the overall literature is inconsistent. A recent paper by Albanes et al[15] found that men with higher 25(OH)D concentrations were at increased risk of developing prostate cancer over up to 20 years follow-up (40-60% increased risk in men with the highest vs lowest levels). They found no evidence of greater risk in men with low vitamin D status. Our analysis found no evidence to replicate these previous findings of an increased risk of prostate cancer with both high and low 25(OH)D levels. The range of total 25(OH)D was comparable between these studies and our data (mean total 25(OH)D ranging from 18.5ng/mL to 24.6mg/mL). Differences in total 25(OH)D across studies may relate to different methodology used to measure total 25(OH)D, or to other factors such as differing latitude or fortification practices between

countries in which the studies were conducted[12]. Our data add to those of Yin et al in suggesting no evidence of a U-shaped relationship.

The overall level of circulating total 25(OH)D (mean=22.9ng/mL) was slightly higher than that reported by the National Diet and Nutrition Survey, designed to be representative of the UK population, which found that the median concentration of plasma 25(OH)D in men aged 50 to 64 years was 20.3ng/mL (SD=8.5; n=190)[31]. This may be method-related or because included men were healthier than the general population in order for them to be eligible for the ProtecT study (as men were excluded due to pre-existing co-morbidities that would rule out surgery or radical radiotherapy). Also, ProtecT includes few men who identified themselves as black or South Asian, who are likely to have lower total 25(OH)D levels[32]. An age-related decline in total 25(OH)D levels is expected by, on average, between 0.3 and 0.6ng/mL per year[33]. We found that vitamin D levels increased with age, which may be because retired men who are healthy have more opportunity for sun exposure[34].

Variation of total 25(OH)D by season of blood draw, with the highest levels of total 25(OH)D occurring in the summer months and the lowest in winter, has been consistently demonstrated[33, 35]. We accounted for seasonal variation in our analysis by matching cases and controls on strata that included date of blood draw. In the case-only analysis, where strata-matching was not accounted for, we adjusted for season of blood draw. Increasing adiposity has consistently been associated with decreasing circulating total 25(OH)D concentrations[33-37], but BMI was not strongly associated with prostate cancer in ProtecT[38] and it did not confound our observed associations.

### Strength and limitations of our study

All of our men were resident in the UK and 99% of our subjects self-reported their ethnicity as white so we cannot assess differences by large changes in latitude or ethnicity. It is also possible that we are studying a relatively healthy population, within which there is not enough variation in total 25(OH)D to be able to ascertain correlations with very high or very low levels. It is widely accepted that total 25(OH)D is the best indicator of short term vitamin D status[39] but measuring circulating total 25(OH)D may not reflect biologically active levels in tissues, since the prostate can locally produce $1,25(OH)_2D$[40]. However, levels of $1,25(OH)_2D$ are tightly regulated and only fluctuate during insufficiency or severe deficiency of total 25(OH)D. $25(OH)D_3$ has a half-life of two to three weeks[33], so a single measurement of total 25(OH)D at study baseline may not reflect vitamin D status over the life-course, which may be most relevant to cancer aetiology[41]. Indeed, prostate cancer risk in UK South Asians is consistent with high sun exposure during young adulthood conferring life-long protection[42]. However, studies assessing the reliability of measurements have shown a low within-person variability over five years (14.9%, 95% CI 12.4,18.1)[43] and a high agreement across time points with intra-class correlation coefficients of 0.7 between samples collected three[28] and five years apart[43]. Serum stored for over 40 years was found to have the same predictive power as serum stored for up to two years[44], indicating that even though our samples were stored for 4.4 years on average, this should not affect our results.

There is potential for residual confounding, as vitamin D status is likely to be a marker of overall health but we have adjusted for the main risk factors for prostate cancer. Since the decision to biopsy was based on PSA level, some of the controls with PSA<3ng/mL will have unidentified prostate cancer[45] (misclassification bias) but this would not affect our analysis of advanced versus localized cancers (as all cancers were biopsy confirmed). Studies have suggested an inverse association between total 25(OH)D and PSA level[46-47], but there was no evidence of an association between PSA level and total 25(OH)D in our data. Therefore any misclassification of cancer status is likely to be non-differential with respect to vitamin D status, at most moderately attenuating any effect-estimates. More

affluent men tend to have PSA tests and thus be diagnosed with prostate cancer, and such men may have higher vitamin D exposure[48] but this would not affect our study where all men had PSA tests. Since the men had prostate cancer at the time of 25(OH)D measurement, it is possible that our results are due to reverse causality (more advanced cases causing lower circulating vitamin D levels). Our case-only comparison uses 25(OH)D levels measured in men diagnosed at the time of their advanced/high-grade cancer versus men diagnosed at the time of their localized/low-grade cancer. We do not have information on 25(OH)D levels prior to disease progression, and therefore cannot rule out reverse causality.

The strengths of our study are the large sample, about which we have extensive information recorded. Circulating total 25(OH)D concentrations were measured at one laboratory, in as few batches and in as short a time frame as possible (thus attenuating any potential technical errors of measurement). The likelihood of falling into the various vitamin D exposure categories is highly dependent on the season of blood draw. However, we know the date of blood draw, and can therefore adjust analyses for season. Recall bias is unlikely since the questionnaire data were predominantly collected prior to the results of the PSA test being known (in 85% of men). The study is population-based and thus subject to little selection bias. Using total 25(OH)D as a biomarker captures vitamin D exposure from sunlight, diet and supplements.

## Conclusion

Our study found evidence that lower total 25(OH)D concentrations were associated with more aggressive cancers amongst cases (advanced versus localized cancers and high- versus low-Gleason grade), but there was no evidence of an association with overall prostate cancer risk. This study adds further evidence to the Cancer Research UK SunSmart recommendations[49], that the limited amount of sun exposure required to obtain adequate vitamin D levels may reduce aggressive prostate cancer. Randomised controlled trials are required to determine whether supplementation or dietary sources of vitamin D would reduce the risk of aggressive prostate cancer or prostate cancer progression.

## Acknowledgments

## Abbreviations

**(25(OH)D)**        25-hydroxyvitamin D

| (ProtecT) | Prostate Testing for Cancer and Treatment trial |
| **(PSA)** | prostate specific antigen |
| **(ng/mL)** | nanograms per millilitre |
| **(OR)** | odds ratios |
| **(CI)** | confidence intervals |
| **(BMI)** | body mass index |
| **(MAP)** | mean arterial pressure |

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## Statements

Our study, of 1,447 prostate cancer cases and 1,449 healthy controls from the ProtecT study, found evidence of a two-fold increased risk of advanced versus localized prostate cancer and high-grade versus low-grade cancer in men deficient in vitamin D. There was no evidence of an association between total 25(OH)D and overall prostate cancer incidence. This study adds further evidence that the modest sun exposure required to obtain adequate vitamin D levels may reduce aggressive disease in men with prostate cancer.
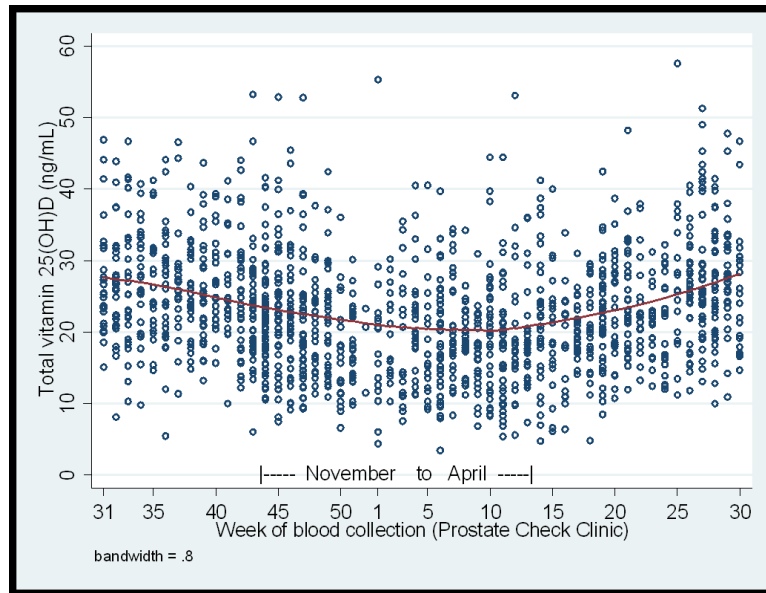
**Figure 1.**
Concentrations of total 25(OH)D by calendar week of blood collection in controls (n=1449). Each circle represents an individual measurement. The smoothed line shows the mean total 25(OH)D values calculated using locally weighted polynomial regression.

**Table 1**

Baseline characteristics of cases and controls included in the study

| | Cases n=1447 | Controls n=1449 | p for difference[a] |
|---|---|---|---|
| **Continuous Variables** | **Mean (SD)** | **Mean (SD)** | |
| **Age (years)** | 62.6 (5.0) | 62.4 (5.0) | 0.26 |
| **PSA (ng/mL)** | 9.4 (26.5) | 1.39 (1.3) | <0.001 |
| **BMI (kg/m$^2$)** | 27.2 (3.6) | 27.3 (3.9) | 0.28 |
| **MAP (mmHg)** | 108.2 (12.4) | 107.1 (12.3) | 0.02 |
| **Storage Time (years)** | 4.3 (1.0) | 4.4 (1.1) | 0.05 |
| **Categorical Variables** | **n (%)** | **n (%)** | **p for heterogeneity** [b] |
| **Age group** | | | |
| 50-54 | 139 (9.61) | 140 (9.66) | |
| 55-59 | 301 (20.80) | 303 (20.91) | |
| 60-64 | 475 (32.83) | 466 (32.16) | |
| >=65 | 532 (36.77) | 540 (37.27) | 0.98 |
| **Centre of Recruitment** | | | |
| Sheffield | 213 (14.72) | 216 (14.91) | |
| Newcastle | 194 (13.41) | 193 (13.32) | |
| Bristol | 130 (8.98) | 132 (9.11) | |
| Cardiff | 147 (10.16) | 148 (10.21) | |
| Edinburgh | 130 (8.98) | 129 (8.90) | |
| Birmingham | 33 (2.28) | 32 (2.21) | |
| Leicester | 264 (18.24) | 265 (18.29) | |
| Cambridge | 162 (11.20) | 163 (11.25) | |
| Leeds | 174 (12.02) | 171 (11.80) | 1.00 |
| **Centre Location** | | | |
| North | 711 (49.14) | 709 (48.93) | |
| South | 736 (50.86) | 740 (11.80) | 0.91 |
| **Season of blood draw** | | | |
| Winter | 327 (22.60) | 337 (23.26) | |
| Spring | 336 (23.22) | 333 (22.98) | |
| Summer | 389 (26.88) | 375 (25.88) | |
| Autumn | 395 (27.30) | 404 (27.88) | 0.91 |
| **Family History** | | | |
| No | 1182 (91.70) | 1234 (94.27) | |
| Yes | 107 (8.30) | 75 ( 5.73) | 0.01 |
| **Social Class** | | | |
| Managerial/professional | 594 (42.40) | 608 (42.55) | |
| Intermediate | 219 (15.63) | 235 (16.45) | |

| | Cases n=1447 | Controls n=1449 | p for difference[a] |
|---|---|---|---|
| **Continuous Variables** | **Mean (SD)** | **Mean (SD)** | |
| Working | 588 (41.97) | 586 (41.01) | 0.80 |
| **BMI** | | | |
| normal 18.5-25 | 335 (28.46) | 307 (28.77) | |
| overweight >=25 | 621 (52.76) | 534 (50.05) | |
| obese >=30 | 221 (18.78) | 226 (21.18) | 0.29 |
| **Smoking** | | | |
| No | 438 (36.32) | 380 (35.02) | |
| Yes | 768 (63.68) | 705 (64.98) | 0.52 |
| **Weekly exercise** | | | |
| None | 550 (46.65) | 481 (45.42) | |
| 1-2 times | 367 (31.13) | 355 (33.52) | |
| 3-4 times | 184 (15.61) | 159 (15.01) | |
| 5+ times | 78 ( 6.62) | 64 ( 6.04) | 0.66 |
| **Diabetes** | | | |
| No | 1045 (94.06) | 936 (91.59) | |
| Yes | 66 ( 5.94) | 86 ( 8.41) | 0.03 |
| **History of BPH** | | | |
| No | 1298 (91.73) | 1272 (89.70) | |
| Possible | 63 ( 4.45) | 84 ( 5.92) | |
| Yes | 54 ( 3.82) | 62 ( 4.37) | 0.15 |

[a]Calculated using a t-test

[b]Calculated using a Chi-squared test ($\chi^2$)

Men in this table had an available 25(OH)D result.

SD=standard deviation; BMI=body mass index; MAP=mean arterial pressure; BPH=benign prostatic hyperplasia

**Table 2**

Mean total 25(OH)D levels (ng/mL) by age group, season of blood draw and study centre location in cases and controls

| | All Prostate Cancer Cases (n=1447) | | | All Controls (n=1449) | | | p for difference[a] |
|---|---|---|---|---|---|---|---|
| | Mean 25(OH)D (ng/mL) | SD | p-value[b] | Mean 25(OH)D (ng/mL) | SD | p-value[b] | |
| **All** | 23.0 | 8.7 | | 22.9 | 8.4 | | 0.72 |
| **Age group** | | | | | | | |
| 50-54 [*] | 22.9 | 8.8 | | 20.9 | 7.9 | | 0.05 |
| 55-59 | 22.3 | 8.4 | 0.53 | 22.7 | 8.8 | 0.04 | 0.63 |
| 60-64 | 22.8 | 8.8 | 0.96 | 23.0 | 8.2 | 0.01 | 0.70 |
| >=65 | 23.7 | 8.8 | 0.31 | 23.5 | 8.3 | 0.001 | 0.71 |
| p for linear trend[c] | | | 0.06 | | | 0.001 | |
| **Centre Location** | | | | | | | |
| North [*] | 23.5 | 9.1 | | 23.7 | 8.4 | | 0.62 |
| South | 22.6 | 8.3 | 0.06[d] | 22.2 | 8.3 | 0.005[d] | 0.31 |
| **Season of blood draw** | | | | | | | |
| Winter [*] | 18.9 | 7.4 | | 19.3 | 8.0 | | 0.50 |
| Spring | 22.4 | 8.4 | <0.001 | 22.4 | 7.6 | <0.001 | 0.94 |
| Summer | 27.0 | 8.8 | <0.001 | 26.9 | 7.9 | <0.001 | 0.76 |
| Autumn | 23.1 | 8.1 | <0.001 | 22.8 | 8.1 | <0.001 | 0.61 |

[*] Reference category

[a] Calculated using a t-test

[b] p-value calculated from regression models, containing total 25(OH)D level and the variable of interest.

[c] p-value for linear trend calculated by treating categorical variable as continuous in regression model.

[d] Regression model additionally adjusted for season of blood draw

SD=standard deviation

**Table 3**

Associations between total 25(OH)D and risk of prostate cancer

| 25(OH)D (ng/mL) | N (%) Cases | N (%) Controls | Adjusted for age and assay batch | | |
| --- | --- | --- | --- | --- | --- |
| | | | OR | 95% CI | p-value[a] |
| High (>=30ng/mL) | 294 (20.3) | 270 (18.6) | 1.05 | (0.84, 1.32) | 0.65 |
| Adequate (20–<30ng/ml) | 574 (39.7) | 616 (42.5) | 1 | | |
| Insufficient (12–<20ng/ml) | 453 (31.3) | 445 (30.7) | 1.14 | (0.93 1.38) | 0.21 |
| Deficient (<12ng/ml) | 126 (8.7) | 118 (8.1) | 1.08 | (0.79, 1.50) | 0.62 |
| p for linear trend[b] | | | | | 0.50 |
| per SD decrease in 25(OH)D (standard deviation = 8.6 ng/mL) | 1449 | 1447 | 1.00 | (0.92, 1.10) | 0.92 |
| p linearity assumption[c] | | | | | 0.56 |

[a] Calculated from regression models.

[b] p-value for linear trend calculated by treating categorical 25(OH)D as continuous, and setting the reference (baseline) category as 'High'.

[c] p-value for a likelihood ratio test of the assumption of linearity. Small p value indicates evidence against linearity.

Model is calculated using conditional logistic regression, stratum matched on 5-year age band and GP surgery and additionally adjusted as indicated. An odds ratio of greater than 1 indicates that the exposure is associated with increased risk of prostate cancer compared to controls. An odds ratio of less than 1 indicates that the exposure is associated with decreased risk of prostate cancer compared to controls.

OR=odds ratio; CI=confidence interval; p=p-value; SD=standard deviation.

**Table 4**

Associations between total 25(OH)D and prostate cancer stage and grade in cases only, and between advanced/high-grade cases versus controls and localised/low-grade cases versus controls

| 25(OH)D (ng/mL) | Advanced N (%) | Localised N (%) | Controls N (%) | Case-only Analysis[a] Adjusted for age and assay batch OR | 95%CI | p-value[c] | Advanced/High-grade cases vs controls Analysis[b] Adjusted for age and assay batch OR | 95%CI | p-value[c] | Localised/Low-grade cases vs controls Analysis[b] Adjusted for age and assay batch OR | 95%CI | p-value[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| High (>=30ng/mL) | 26 (17.0) | 268 (20.8) | 270 (18.6) | 0.83 | (0.50, 1.40) | 0.49 | 0.92 | (0.48, 1.76) | 0.81 | 1.08 | (0.85, 1.37) | 0.39 |
| Adequate (20-<30ng/ml) | 56 (36.6) | 516 (40.0) | 616 (42.5) | 1 | | | 1 | | | 1 | | |
| Insufficient (12-<20ng/ml) | 50 (32.7) | 400 (31.0) | 445 (30.7) | 1.17 | (0.76, 1.80) | 0.47 | 1.43 | (0.81, 2.50) | 0.22 | 1.10 | (0.89, 1.35) | 0.39 |
| Deficient (<12ng/ml) | 21 (13.7) | 105 (8.2) | 118 (8.1) | 2.30 | (1.27, 4.19) | 0.01 | 1.44 | (0.64, 3.24) | 0.38 | 1.00 | (0.71, 1.39) | 0.98 |
| p for linear trend[d] | | | | | | 0.01 | | | 0.96 | | | 0.57 |
| per SD decrease in 25(OH)D (standard deviation =8.6 ng/mL) | 153 | 1289 | 1449 | 1.29 | (1.06, 1.57) | 0.01 | 1.24 | (0.96, 1.59) | 0.10 | 0.97 | (0.89, 1.07) | 0.56 |
| p linearity assumption[e] | | | | | | 0.39 | | | | | | |

| 25(OH)D (ng/mL) | High-grade N (%) | Low-grade N (%) | Controls N (%) | Case-only Analysis[a] Adjusted for age and assay batch OR | 95%CI | p-value[c] | Advanced/High-grade cases vs controls Analysis[b] Adjusted for age and assay batch OR | 95%CI | p-value[c] | Localised/Low-grade cases vs controls Analysis[b] Adjusted for age and assay batch OR | 95%CI | p-value[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| High (>=30ng/mL) | 97 (20.7) | 195 (20.0) | 270 (18.6) | 1.21 | (0.87, 1.67) | 0.25 | 1.21 | (0.85, 1.71) | 0.29 | 1.05 | (0.80, 1.37) | 0.73 |
| Adequate (20-<30ng/ml) | 164 (35.0) | 408 (41.9) | 616 (42.5) | 1 | | | 1 | | | 1 | | |
| Insufficient (12-<20ng/ml) | 157 (33.5) | 296 (30.4) | 445 (30.7) | 1.35 | (1.01, 1.78) | 0.04 | 1.41 | (1.03, 1.92) | 0.03 | 1.04 | (0.83, 1.31) | 0.75 |
| Deficient (<12ng/ml) | 51 (10.9) | 75 (7.7) | 118 (8.1) | 1.82 | (1.18, 2.81) | 0.01 | 1.48 | (0.90, 2.42) | 0.12 | 0.96 | (0.66, 1.39) | 0.83 |
| p for linear trend[d] | | | | | | 0.05 | | | 0.18 | | | 0.81 |
| per SD decrease in 25(OH)D (standard deviation =8.6 ng/mL) | 469 | 974 | 1449 | 1.14 | (1.00, 1.29) | 0.04 | 1.11 | (0.97, 1.28) | 0.14 | 0.96 | (0.86, 1.06) | 0.39 |
| p linearity assumption[e] | | | | | | 0.08 | | | | | | |

[a] Case-only models are calculated using logistic regression, adjusted for season of blood draw and study centre location. Models are additionally adjusted as indicated.

[b] Case vs control models are calculated using conditional logistic regression, stratum matched on 5-year age band and GP surgery. Models additionally included exact age and assay batch.

[c] Calculated from regression models.

[d] p-value for linear trend calculated by treating categorical 25(OH)D as continuous, and setting the reference (baseline) category as 'High'.

[e] p-value for a likelihood ratio test of the assumption of linearity. Small p value indicates evidence against linearity.

Advanced stage is T3-T4, localized stage is T1-T2, N0, M0. High-grade is Gleason grade ≥7, low-grade is <7.

An odds ratio of greater than 1 indicates that the exposure is associated with increased risk of advanced cancer compared to localised cancer, or high-grade cancer compared with low-grade cancer. An odds ratio of less than 1 indicates that the exposure is associated with decreased risk of advanced cancer compared to localised cancer, or high-grade cancer compared with low-grade cancer.

OR=odds ratio; CI=confidence interval ; p=p-value; SD=standard deviation.