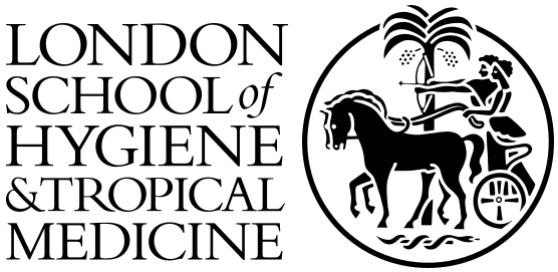

A collaborative analysis of individual participant data from 19 prospective studies assesses circulating vitamin D and prostate cancer risk

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the writing team had full access to all data in the study. The corresponding author had the final responsibility for the decision to submit for publication. The EHNBPCCG data are not suitable for sharing, but are suitable for research involving further analyses in collaboration with the Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford.

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Abbreviations: CI, confidence interval; EHNBPCCG, Endogenous Hormones, Nutritional Biomarkers and Prostate Cancer Collaborative Group; IGF-I, insulin-like growth factor-I; IGFBP3, IGF binding protein-3; OR, odds ratio; PSA, prostate-specific antigen; SHBG, sex hormone-binding globulin; TNM, tumor-node-metastasis; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D.

Abstract

Previous prospective studies assessing the relationship between circulating concentrations of vitamin D and prostate cancer risk have shown inconclusive results, particularly for risk of aggressive disease. In this study, we examine the association between pre-diagnostic concentrations of 25-hydroxyvitamin D (25(OH)D) and $1,25(OH)_2D$ and the risk of prostate cancer overall and by tumor characteristics. Principal investigators of 19 prospective studies provided individual participant data on circulating 25(OH)D and $1,25(OH)_2D$ for up to 13,462 men with incident prostate cancer and 20,261 control participants. Odds ratios (OR) for prostate cancer by study-specific fifths of season-standardized vitamin D concentration were estimated using multivariable-adjusted conditional logistic regression. 25(OH)D concentration was positively associated with risk for total prostate cancer (multivariable-adjusted OR comparing highest versus lowest study-specific fifth was 1.22, 95% CI 1.13-1.31; P trend<0.001). However, this association varied by disease aggressiveness ($P_{heterogeneity}$=0.014); higher circulating 25(OH)D was associated with a higher risk of non-aggressive disease (OR per 80 percentile increase=1.24, 1.13-1.36) but not with aggressive disease (defined as stage 4, metastases, or prostate cancer death, 0.95, 0.78-1.15). $1,25(OH)_2D$ concentration was not associated with risk for prostate cancer overall or by tumor characteristics. The absence of an association of vitamin D with aggressive disease does not support the hypothesis that vitamin D deficiency increases prostate cancer risk. Rather, the association of high circulating 25(OH)D concentration with a higher risk of non-aggressive prostate cancer may be influenced by detection bias.

Statement of significance

This international collaboration comprises the largest prospective study on blood vitamin D and prostate cancer risk and shows no association with aggressive disease but some evidence of a higher risk of non-aggressive disease.

Introduction

It has been hypothesized that vitamin D deficiency may increase prostate cancer risk (1,2). A meta-analysis of 6 prospective studies published up to 2010 reported that circulating vitamin D concentrations were not related to prostate cancer risk (3); however, it was insufficiently powered to provide robust estimates of risk, especially for important disease subgroups. While the active hormonal form of vitamin D is 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which is mainly formed by hydroxylation of 25-hydroxyvitamin D (25(OH)D) in the kidney under the control of parathyroid hormone, circulating 25(OH)D concentration is regarded as the most informative indicator of vitamin D status.

The Endogenous Hormones, Nutritional Biomarkers and Prostate Cancer Collaborative Group (EHNBPCCG) was established to conduct collaborative reanalyzes of individual data from prospective studies on the relationships of circulating hormone concentrations and nutritional biomarkers with prostate cancer risk (4,5). With pooled individual participant data on pre-diagnostic circulating 25(OH)D and $1,25(OH)_2D$ concentrations from 19 prospective studies (with up to 13,462 men with incident prostate cancer), this analysis aimed to provide precise estimates of the association of circulating vitamin D with prostate cancer risk and to investigate whether these associations differed by tumor characteristics or time from blood collection to diagnosis. We also examined the cross-sectional relationships between lifestyle factors and vitamin D concentrations.

Material and methods

Data collection

Published and unpublished studies were eligible for the current analysis if they had data on pre-diagnostic circulating concentrations of 25(OH)D or $1,25(OH)_2D$ and incident prostate cancers.

Studies were identified using literature search methods from computerized bibliographic systems and by discussion with collaborators, as described previously (4,5). Data were available for 19 prospective studies by dataset closure in May 2018.

Individual participant data were requested on circulating 25(OH)D and $1,25(OH)_2D$, date, age and fasting status at sample collection, marital status, ethnicity, educational attainment, family history of prostate cancer, height, weight, waist and hip circumference, smoking status, alcohol intake, and vital status. Each study also provided data on prostate cancer stage and grade and death, if available, and the data were harmonized in a central database. Further details on data collection and processing are provided in the **Supplementary Methods**.

Study designs and data processing

The characteristics of the included studies are shown in **Supplementary Table 1** and details of the assay methods are shown in **Supplementary Table 2**. Most of the studies were case-control studies nested within prospective cohort studies. Data on the control participants from The Prostate Testing for Cancer and Treatment (ProtecT) trial are included in cross-sectional analyses of vitamin D concentrations in relation to participant characteristics, but because cases were diagnosed at the start of the study rather than during follow-up, these data were not included in the main risk analyses. Written informed consent was obtained from study participants at entry into each cohort or was implied by participants' return of the enrolment questionnaire. The study protocols were approved by institutional review boards of each study center.

Prostate cancer was defined as being 'early' stage if it was tumor-node-metastasis (TNM) stage T1 with no reported lymph node involvement or metastases, or stage I; 'other localized' stage if it was TNM stage T2 with no reported lymph node involvement or metastases, stage II, or the equivalent; 'advanced' stage if it was TNM stage T3 or T4 and/or N1+ and/or M1, stage III–IV, or the equivalent; or stage unknown. Aggressive disease was categorized as "no" for TNM stage T0,

T1, T2 or T3 with no reported lymph node involvement and no metastases or equivalent, “yes” for TNM stage T4 and/or N1+ and/or M1 and/or stage IV disease and/or death from prostate cancer, or “unknown”. Histological grade was defined as ‘low-intermediate’ if the Gleason sum was < 8 or equivalent, ‘high’ grade if the Gleason sum was ≥ 8 or equivalent, or grade “unknown”. Fatal cases were men who died of prostate cancer during follow-up.

Statistical analyses

25(OH)D and 1,25$(OH)_2$D concentrations were log-transformed to approximate a normal distribution for parametric analyses. To allow for the influence of month of blood draw on circulating concentrations, a regression model of log-transformed vitamin D concentration by month of blood collection was fitted for each study. All results are presented by season-standardized vitamin D, unless otherwise specified.

The main method of analysis was logistic regression conditioned on the matching variables within each study. Men were categorized into fifths of the distribution of 25(OH)D and 1,25$(OH)_2$D, with cut-points defined by the study-specific quintiles of the distribution within control participants, to allow for any systematic differences between the studies in assay methods and blood sample types (6). Linear trends were calculated by replacing the categorical variable representing the fifths of each analyte with a continuous variable that was scored as 0, 0.25, 0.5, 0.75, and 1; a unit increase in this variable can be taken to represent an 80 percentile increase in the study-specific concentration of vitamin D. To examine the effects of potential confounders (other than the matching criteria, which were taken into account in the study design and matched analyses), conditional logistic regression analyses included the following covariates: age at blood collection, body mass index (BMI), height, marital status, educational status, and cigarette smoking, all of which were associated with prostate cancer risk in these analyses.

In a sensitivity analysis, conditional logistic regression models were also fitted using quintile cut-points defined by the overall distribution among the control participants in all studies combined. The analyses were also repeated using predefined categories for concentrations of 25(OH)D of <30, 30-<50, 50-<75 and ≥75 nmol/L, in order to investigate risks associated with very low (deficiency), low (insufficiency), moderate (sufficiency) and high circulating concentrations of vitamin D based on the Institute of Medicine recommendations (7).

For each analyte, heterogeneity in linear trends between studies was assessed by comparing the χ^2 values for models with and without a (study) x (linear trend) interaction term. Tests for heterogeneity for the case-defined factors were obtained by fitting separate models for each subgroup and assuming independence of the ORs using a method analogous to a meta-analysis, in which controls in each matched set were assigned to the category of their matched case. Tests for heterogeneity for non-case defined factors were assessed with χ^2 tests of interaction between subgroups and the binary variable.

In order to assess potential effect modification with different biomarkers, a χ^2 test of interaction was used to determine whether risks by study-specific thirds of 25(OH)D varied according to study-specific thirds of $1,25(OH)_2D$ (and vice versa), and according to study-specific thirds of circulating concentrations of insulin-like growth factor-I (IGF)-I, IGF binding protein-3 (IGFBP3), testosterone, free testosterone, sex hormone-binding globulin (SHBG) and prostate-specific antigen (PSA), where these data were available.

The cross-sectional associations of 25(OH)D and $1,25(OH)_2D$ with participant characteristics (among controls only) were examined using analyses of variance to calculate geometric mean concentrations and 95% confidence intervals (CIs), adjusting for study and age at blood collection, as appropriate.

All tests of statistical significance were two-sided, and statistical significance was set at the 5% level. All statistical tests were carried out with Stata Statistical Software, Release 14 (StataCorp, LP, College Station, Texas). Full details of the statistical analyses are provided in the **Supplementary Methods**.

Results

Details of the 19 participating studies are shown in **Table 1**. Data on 25(OH)D concentrations were available for 13,462 men who subsequently developed prostate cancer and 20,261 control participants, and for 1,25$(OH)_2$D concentrations for 1,885 case and 2,114 control participants. Mean age at blood collection across the studies ranged from 46.5 (SD = 4.2) to 76.3 (3.6) years. Blood collection preceded prostate cancer diagnosis by an average of 8.5 years (SD = 6.0 years), although there was a wide variation among the studies (**Table 2**). On average, cases were 67.5 years old (SD = 7.3 years) at diagnosis and most (87.1%) were diagnosed after 1994. The majority of cases with information on stage and grade of disease had localized (early or other localized) disease (ranging from 47.8% to 99.0% of case patients across studies) and low-intermediate grade tumors (ranging from 75.8% to 100% of case patients). Concentrations of 25(OH)D and 1,25$(OH)_2$D varied significantly by month among both the cases and controls (**Supplementary Figure 1**).

Associations between circulating vitamin D concentrations and prostate cancer risk

25(OH)D concentration was linearly positively associated with risk for total prostate cancer (**Figure 1**); the multivariate-OR for prostate cancer for men in the highest compared with the lowest study-specific fifth was 1.22 (95% CI 1.13 to 1.31; P trend < 0.001). The association was similar when only the matching factors were taken into account (**Supplementary Figure 2**) and there was no evidence of heterogeneity between the contributing studies (**Figure 2A**). When 25(OH)D was

categorized into study-specific tenths, the OR for the highest versus the lowest tenth was 1.34 (1.20 to 1.49; P trend <0.001, **Supplementary Table 3**).

There was no evidence of an association between $1,25(OH)_2D$ concentration and risk for total prostate cancer (see **Figures 1 and 2B**). The association was similar when only the matching-factors were taken into account (**Supplementary Figure 2**).

In sensitivity analyses that used overall quintile cut-points of 25(OH)D across all studies combined (rather than study-specific cut-points), the ORs for total prostate cancer were materially unchanged (**Supplementary figure 3**). When the analyses were repeated using predefined cut-points for 25(OH)D, multivariable-adjusted ORs for total prostate cancer were 0.84 (0.76-0.93), 0.89 (0.84-0.95) and 1.07 (1.00-1.13), respectively, for men with 25(OH)D <30 (at risk for deficiency), 30-49 and ≥75 nmol/L compared to those with concentrations of 50-74 nmol/L (**Supplementary Table 4**).

While there was no evidence of heterogeneity in the association of 25(OH)D with risk by stage of disease, there were differences by disease aggressiveness (P heterogeneity = 0.014): the OR for an 80-percentile increase in 25(OH)D was 1.24, 1.13-1.36 for non-aggressive disease (T1-T3/N0/M0) and 0.95, 0.78-1.15 for aggressive disease (T4, N1, M1 and/or fatal prostate cancer). Similar differences were also seen between low-intermediate and high-grade disease, although these differences were not statistically significant (**Figure 3**). There was no association between circulating 25(OH)D concentrations and fatal prostate cancer (**Figure 3**). **Supplementary Figure 4** shows results from categorical analyses of the associations of study-specific fifths of 25(OH)D with risk for advanced stage, aggressive disease and high-grade prostate cancer.

There was no evidence of heterogeneity in risk of total prostate cancer associated with 25(OH)D according to time to diagnosis or other participant characteristics (**Figure 3**), including season of blood draw (**Figure 4A**) or by circulating concentrations of $1,25(OH)_2D$, IGF-I, IGFBP-3, testosterone, free testosterone, SHBG or PSA (**Supplementary Table 5A to 5G**).

For $1,25(OH)_2D$, there was no evidence of heterogeneity by season of blood draw (**Figure 4B**), time to diagnosis or other tumor characteristics (**Supplementary Figure 5**). There was some evidence of heterogeneity by family history of prostate cancer, with a positive association for men with a positive family history of the disease ($P_{heterogeneity}=0.03$; multivariable-adjusted OR for an 80 percentile increase = 2.26, 95% CI 1.19-4.32, **Supplementary Figure 4**), although this was based on small numbers. There was no evidence of heterogeneity by season of blood draw (**Figure 4**).

Vitamin D concentrations in relation to other participant and sample characteristics

Concentrations of 25(OH)D and $1,25(OH)_2D$ were significantly but not strongly correlated with each other ($r = 0.13$, $p < 0.001$). In the subset of control participants with data available on other analytes, circulating 25(OH)D concentration was weakly correlated with sex hormones and other analytes (**Supplementary Table 6**), but neither 25(OH)D nor $1,25(OH)_2D$ concentration was correlated with PSA ($r = 0.01$ for both). After adjustment for age, 25(OH)D concentration was lower in men who were obese, current smokers, poorly educated, unmarried and non-drinkers (**Figure 5**). $1,25(OH)_2D$ displayed generally similar associations (**Supplementary Figure 6**).

Discussion

This collaborative analysis of individual participant data does not support the hypothesis that vitamin D deficiency and/or insufficiency increases the risk of prostate cancer. Higher 25(OH)D levels were associated with an increased risk of non-aggressive disease, with no association for aggressive disease. We also found no evidence that circulating concentration of $1,25(OH)_2D$ was related to risk for prostate cancer, overall or by tumor characteristics.

This collaborative analysis includes information from the vast majority (>90%) for 25(OH)D and 85% for $1,25(OH)_2D$ of the published prospective data. Of the 24 studies with published data on

25(OH)D, seven did not contribute data to this collaboration, all of which had fewer than 200 incident cases and reported inconsistent findings (8-13). Combining the results of the current analyses with those of six of the seven additional studies (for whom data could be extracted to perform a meta-analysis), did not change the overall finding (summary relative risk of highest compared with the lowest fifth of 25(OH)D = 1.21, 95% CI 1.13-1.30), suggesting that inclusion of participant-level data from these studies would not have materially altered the results. Two studies with published data on $1,25(OH)_2D$ did not contribute data, one of which reported an inverse association (based on 181 cases, RR not given for $1,25(OH)_2D$ alone) (10,14) and another that found no association (based on 136 cases) (9). Including these two studies would not have materially changed our results. Thus, we believe that the findings from the current study provide a reliable summary of the totality of the evidence on the association between circulating vitamin D concentrations and prostate cancer risk.

Our findings do not appear to support the evidence from experimental research using cell lines and animal models that vitamin D compounds may promote cell differentiation, inhibit prostate cancer cell growth and invasion, and stimulate apoptosis (15,16). While there are no published data from adequately powered randomized controlled trials for the effects of vitamin D supplementation on prostate cancer incidence, two large recent studies have exploited GWAS-identified variation in genes related to vitamin-D synthesis, metabolism and binding to study the possible relationship with prostate cancer risk. A Mendelian randomization analysis of data from up to 69,837 prostate cancer cases in the PRACTICAL and GAME-ON consortia found no evidence for an association with risk for either total (OR in PRACTICAL per genetically-determined 25 nmol/L increase in 25(OH)D concentration = 0.95, 95% CI 0.80-1.13; P = 0.55) or aggressive prostate cancer (OR in GAME-ON = 1.14, 0.85-1.54; P = 0.38) (17).

It is possible that our finding of a positive association between overall and non-aggressive prostate cancer risk and circulating 25(OH)D concentration may be explained by detection bias, in that health-conscious men who may be more likely to have a higher sun exposure, a higher dietary intake of vitamin D and/or vitamin D supplementation, are more likely to have a PSA test or to seek medical attention with early symptoms. The observation that vitamin D deficiency was associated with a reduced risk of prostate cancer and higher levels with an increased risk (particularly for non-aggressive disease) supports this hypothesis. Nonetheless, a positive association between 25(OH)D and prostate cancer risk was reported in both the PLCO and PCPT studies, in which almost all men had either regular PSA testing (as data were provided solely from the screening arm in PLCO and PCPT) or had an end-of-study biopsy (PCPT), suggesting that factors other than detection bias may be involved.

It is difficult to draw conclusions from the current pooled analyses of $1,25(OH)_2D$ as only a small number of prospective studies have measured this analyte. While circulating $1,25(OH)_2D$ concentrations are considered to be tightly regulated within a narrow range (18), we found some evidence of seasonal variation in $1,25(OH)_2D$ concentrations, similar to that of 25(OH)D, and also differences in concentrations according to age, adiposity, cigarette smoking status and alcohol consumption. It is difficult to determine the extent to which these associations are due to cross-reactivity of the $1,25(OH)_2D$ assay with 25(OH)D (or other molecules), although the correlation between 25(OH)D and $1,25(OH)_2D$ was weak ($r=0.13$) and there was no evidence for an association between $1,25(OH)_2D$ and prostate cancer risk.

A number of previous studies have evaluated the joint association of 25(OH)D and $1,25(OH)_2D$ with prostate cancer risk (9,19-21), but their sample sizes were small. We found no evidence that the association of prostate cancer risk with 25(OH)D is modified by circulating concentrations of $1,25(OH)_2D$, although even in this collaborative pooled dataset, there are still

relatively few cases (n=1,885) with data on both vitamin D analytes. It has also been hypothesized that vitamin D may influence tumor growth by modulating the action of growth factors, such as IGF-I, that normally stimulate proliferation (16), for example by stimulating the release of IGFBP-3 (22). We observed weak correlations of circulating 25(OH)D or $1,25(OH)_2D$ concentrations with IGF-I, IGFBP-1, IGFBP-2 and IGFBP-3 concentrations and with levels of other blood biomarkers (e.g. free testosterone or PSA), and there was no evidence of modification of the association of 25(OH)D with risk according to these biomarkers.

This study has some limitations. The calculated relative risks were based on single measurements of vitamin D, which may not accurately reflect long-term circulating concentration. Several studies have found moderate correlations between two measures of 25(OH)D, even when the samples were not taken at the same time of the year, with correlations between 0.42 and 0.70 in blood taken between 3 to 14 years apart (reviewed in (23)). These findings suggest that a single measure of circulating 25(OH)D is an informative measure of vitamin D status, at least over the medium term. The published prospective data on vitamin D and risk for aggressive prostate cancer subtypes are still relatively limited. Thus, even in this pooled analysis, the total number of cases with aggressive disease and data on 25(OH)D is relatively small (n=1,446), therefore the results by tumor sub-type should be interpreted with some caution. Moreover, we don't have detailed data on other sun exposure measures, such as solar radiation levels in each study location, which would also vary within each individual study depending on where each participant lives. Finally, more than 95% of participants included in this pooled analysis were of White ethnicity, and results may therefore not be generalizable to non-White populations.

In summary, this collaborative analysis of the worldwide data on circulating vitamin D and prostate cancer risk suggests that a high vitamin D concentration is not associated with a lower risk of prostate cancer. Rather, the findings suggest that men with elevated circulating concentrations of

25(OH)D are more likely to be diagnosed with non-aggressive prostate cancer, though this may be due to detection bias. There was no evidence for an association with aggressive disease, suggesting that vitamin D is not casually related to the risk of prostate cancer.

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References

1. Schwartz GG, Hanchette CL. UV, latitude, and spatial trends in prostate cancer mortality: all sunlight is not the same (United States). Cancer Causes Control 2006;17:1091-101

2. Schwartz GG, Hulka BS. Is vitamin D deficiency a risk factor for prostate cancer? (Hypothesis). Anticancer Res 1990;10:1307-11

3. Gilbert R, Martin RM, Beynon R, Harris R, Savovic J, Zuccolo L, et al. Associations of circulating and dietary vitamin D with prostate cancer risk: a systematic review and dose-response meta-analysis. Cancer Causes Control 2011;22:319-40

4. Roddam AW, Allen NE, Appleby P, Key TJ, Endogenous H, Prostate Cancer Collaborative G. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst 2008;100:170-83

5. Price AJ, Travis RC, Appleby PN, Albanes D, Barricarte Gurrea A, Bjorge T, et al. Circulating Folate and Vitamin B and Risk of Prostate Cancer: A Collaborative Analysis of

Individual Participant Data from Six Cohorts Including 6875 Cases and 8104 Controls. Eur Urol 2016

6. Key TJ, Appleby PN, Allen NE, Reeves GK. Pooling biomarker data from different studies of disease risk, with a focus on endogenous hormones. Cancer Epidemiol Biomarkers Prev 2010;19:960-5

7. In: Ross AC, Taylor CL, Yaktine AL, Del Valle HB, editors. Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC)2011.

8. Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, Hallmans G, et al. Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer 2004;108:104-8

9. Nomura AM, Stemmermann GN, Lee J, Kolonel LN, Chen TC, Turner A, et al. Serum vitamin D metabolite levels and the subsequent development of prostate cancer (Hawaii, United States). Cancer Causes Control 1998;9:425-32

10. Corder EH, Guess HA, Hulka BS, Friedman GD, Sadler M, Vollmer RT, et al. Vitamin D and prostate cancer: a prediagnostic study with stored sera. Cancer Epidemiol Biomarkers Prev 1993;2:467-72

11. Barnett CM, Nielson CM, Shannon J, Chan JM, Shikany JM, Bauer DC, et al. Serum 25-OH vitamin D levels and risk of developing prostate cancer in older men. Cancer Causes Control 2010;21:1297-303

12. Jacobs ET, Giuliano AR, Martinez ME, Hollis BW, Reid ME, Marshall JR. Plasma levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and the risk of prostate cancer. J Steroid Biochem Mol Biol 2004;89-90:533-7

13. Ordonez-Mena JM, Schottker B, Fedirko V, Jenab M, Olsen A, Halkjaer J, et al. Pre-diagnostic vitamin D concentrations and cancer risks in older individuals: an analysis of cohorts participating in the CHANCES consortium. Eur J Epidemiol 2016;31:311-23

14. Corder EH, Friedman GD, Vogelman JH, Orentreich N. Seasonal variation in vitamin D, vitamin D-binding protein, and dehydroepiandrosterone: risk of prostate cancer in black and white men. Cancer Epidemiol Biomarkers Prev 1995;4:655-9

15. Swami S, Krishnan AV, Feldman D. Vitamin D metabolism and action in the prostate: implications for health and disease. Mol Cell Endocrinol 2011;347:61-9

16. Fleet JC. Molecular actions of vitamin D contributing to cancer prevention. Mol Aspects Med 2008;29:388-96

17. Dimitrakopoulou VI, Tsilidis KK, Haycock PC, Dimou NL, Al-Dabhani K, Martin RM, et al. Circulating vitamin D concentration and risk of seven cancers: Mendelian randomisation study. BMJ 2017;359:j4761

18. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81

19. Gann PH, Ma J, Hennekens CH, Hollis BW, Haddad JG, Stampfer MJ. Circulating vitamin D metabolites in relation to subsequent development of prostate cancer. Cancer Epidemiol Biomarkers Prev 1996;5:121-6

20. Li H, Stampfer MJ, Hollis JB, Mucci LA, Gaziano JM, Hunter D, et al. A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and prostate cancer. PLoS Med 2007;4:e103

21. Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control 2004;15:255-65

22. Peng L, Malloy PJ, Feldman D. Identification of a functional vitamin D response element in the human insulin-like growth factor binding protein-3 promoter. Mol Endocrinol 2004;18:1109-19

23. Meng JE, Hovey KM, Wactawski-Wende J, Andrews CA, Lamonte MJ, Horst RL, et al. Intraindividual variation in plasma 25-hydroxyvitamin D measures 5 years apart among postmenopausal women. Cancer Epidemiol Biomarkers Prev 2012;21:916-24

Table 1. Participant characteristics by study and case-control status[a]

Prospective studies (First author, year)	Case-control status	Number	Age at recruitment (y)	BMI (kg/m^2)	Married or cohabiting (%)[b]	Higher education (%)[b]	Current smoker (%)[b]	Intake of alcohol (g/d)	Family history of prostate cancer (%)[b]	Geometric mean concentration (95% CI)	
										25(OH)D[c] (nmol/L)	1,25(OH)$_2$D[c] (pmol/L)
ARIC (Unpublished)[d]	Case	700	55.4 (5.7)	27.5 (4.0)	88.3	17.9	20.6	9.1 (15.9)	13.3	60.1 (58.6-61.7)	-
	Control	2752	55.2 (5.7)	27.5 (4.2)	85.8	11.6	26.9	9.9 (18.3)	6.2	59.7 (58.8-60.5)	-
ATBC (Albanes *et al.*, 2011)	Case	996	58.4 (5.2)	26.3 (3.6)	82.5	6.1	100	17.3 (23.2)	7.3	32.6 (31.4-33.8)	-
	Control	996	58.4 (5.1)	26.1 (3.7)	81.4	4.3	100	15.4 (19.5)	3.5	31.4 (30.2-32.6)	-
CLUE 1 (Braun *et al.*, 1995)	Case	61	58.3 (8.5)	-	91.8	11.5	29.5	-	-	82.1 (76.3-88.2)	94 (87-102)
	Control	122	58.3 (8.5)	-	86.9	9.8	25.4	-	-	79.0 (74.7-83.4)	91 (85-97)
EPIC (Travis *et al.*, 2010)	Case	652	60.4 (6.3)	26.7 (3.4)	88.8	24.9	20.3	19.1 (23.9)	-	53.8 (52.3-55.3)	-
	Control	752	59.9 (6.3)	26.8 (3.5)	88.6	19.8	22.6	17.0 (19.9)	-	53.2 (51.8-54.7)	-
ESTHER (Ordonez-Mena *et al.*, 2013)	Case	216	64.3 (5.1)	27.3 (3.1)	83.9	-	13.9	16.6 (19.7)	5.1	55.3 (51.7-59.1)	-
	Control	841	64.3 (5.1)	28.0 (4.2)	84.6	-	14.6	14.2 (15.3)	3.8	54.2 (52.6-55.8)	-
FMC (Unpublished)[d]	Case	161	57.9 (10.4)	25.8 (3.1)	90.6	-	29.0	-	-	51.5 (47.6-55.6)	-
	Control	286	57.2 (10.4)	26.1 (3.6)	85.0	-	34.9	-	-	50.4 (47.8-53.0)	-
HIMS (Wong *et al.*, 2014)	Case	332	76.4 (3.7)	26.4 (3.5)	86.7	22.9	4.5	11.7 (15.5)	-	66.8 (64.5-69.2)	-
	Control	1317	76.3 (3.6)	26.5 (3.7)	86.2	21.7	4.6	11.8 (16.1)	-	64.3 (63.1-65.5)	-
HPFS (Platz *et al.*, 2004; Mikkah *et al.*, 2007; Shui *et al.*, 2012)	Case	1326	63.8 (7.8)	26.0 (3.3)	92.7	100	4.4	11.8 (15.4)	14.4	68.1 (66.5-69.7)	83 (81-86)
	Control	1326	63.7 (7.8)	26.1 (3.5)	93.0	100	3.5	11.6 (15.8)	10.6	66.2 (64.4-68.0)	83 (81-85)
Janus part 1 (Tuohimaa *et al.*, 2004)	Case	575	46.5 (4.3)	25.4 (3.1)	-	-	60.6	-	-	52.1 (50.6-53.7)	-
	Control	2233	46.5 (4.2)	25.1 (3.2)	-	-	62.3	-	-	49.7 (49.0-50.4)	-
Janus part 2 (Meyer *et al.*, 2013)	Case	2106	47.7 (9.2)	25.5 (3.0)	-	-	32.8	-	-	60.4 (59.6-61.3)	-
	Control	2106	47.7 (9.2)	25.6 (3.0)	-	-	34.5	-	-	58.7 (57.9-59.6)	-
JPHC (Sawada *et al.*, 2017)	Case	201	59.5 (6.4)	23.4 (2.4)	100	-	34.3	26.9 (31.7)	0.5	86.9 (82.7-91.3)	-
	Control	402	59.2 (6.6)	23.3 (2.6)	100	-	40.8	31.6 (47.6)	0.0	85.6 (82.7-88.6)	-
MCCS (Unpublished)[d]	Case	818	58.4 (7.4)	27.1 (3.4)	82.7	30.7	8.3	17.9 (22.8)	-	52.5 (51.3-53.8)	-
	Control	1151	56.4 (7.7)	26.9 (3.4)	78.2	26.9	12.9	17.8 (23.9)	-	50.2 (49.1-51.2)	-
MDCS (Brandstedt *et al.*, 2012)	Case	910	61.3 (6.4)	26.3 (3.3)	77.8	14.6	22.2	14.9 (14.6)	-	83.4 (81.8-85.0)	-
	Control	910	61.1 (6.4)	26.1 (3.3)	75.7	12.6	26.7	14.6 (14.2)	-	82.0 (80.4-83.6)	-
MEC (Park *et al.*, 2010)	Case	329	68.9 (7.1)	26.6 (4.0)	77.1	34.0	14.1	23.3 (44.1)	13.9	77.6 (74.2-81.2)	-
	Control	656	68.7 (7.2)	26.8 (4.0)	78.6	32.9	12.6	22.5 (39.1)	8.8	75.6 (73.2-78.0)	-

PCPT (Schenk *et al.*, 2014)	Case	915	63.3 (5.5)	27.5 (4.2)	87.5	38.5	6.7	9.6 (15.8)	21.7	58.6 (57.2-60.0)	-
	Control	915	63.3 (5.5)	27.6 (4.0)	87.2	37.7	6.8	8.9 (13.7)	21.6	56.0 (54.7-57.4)	-
PHS (Gann *et al.*, 1996; Ma *et al.*, 1998; Li *et al.*, 2007)	Case	501	58.6 (7.6)	24.6 (2.5)	-	100	7.8	7.2 (6.0)	-	72.6 (70.3-75.0)	79 (77-80)
	Control	669	59.1 (7.6)	24.6 (2.5)	-	100	7.0	7.1 (6.3)	-	71.3 (69.3-73.3)	79 (77-80)
PLCO (Ahn *et al.*, 2008)	Case	747	64.8 (5.0)	27.3 (3.6)	88.0	43.3	6.4	15.7 (29.5)	12.3	56.1 (54.8-57.4)	-
	Control	727	64.5 (4.9)	27.6 (3.9)	85.8	39.5	9.8	16.2 (30.1)	5.2	54.0 (52.7-55.4)	-
SELECT (Kristal *et al.*, 2014)	Case	1732	63.5 (6.1)	28.5 (4.3)	84.1	54.9	5.4	9.4 (15.7)	31.2	64.9 (63.6-66.3)	-
	Control	1732	63.6 (6.4)	28.7 (4.7)	82.6	51.0	7.1	9.2 (20.0)	15.3	63.8 (62.5-65.2)	-
SU.VI.MAX (Deschasaux *et al.*, 2016)	Case	184	54.1 (4.8)	25.5 (3.1)	93.3	30.2	11.1	25.1 (19.2)	12.7	44.1 (41.2-47.2)	-
	Control	368	53.8 (4.4)	25.7 (3.2)	89.3	26.8	12.8	25.5 (18.9)	4.4	45.9 (43.7-48.2)	-

[a] Values are mean (SD) unless otherwise indicated. Numbers are for men with a 25(OH)D measurement and in complete matched case-control sets.

[b] Percentages exclude men with missing values.

[c] 25(OH)D and 1,25(OH)D concentrations are season-standardised.

[d] Unpublished vitamin D and prostate cancer data, Study references: Joshu *et al*., 2018 for ARIC, Knekt *et al.*, 2008 for FMC and Milne *et al*., 2017 for MCCS

Abbreviations: ARIC, Atherosclerosis Risk in Communities Study; ATBC, Alpha-Tocopherol Beta-Carotene Cancer Prevention Study; BMI, body mass index; CLUE, Campaign Against Cancer and Stroke ("Give Us a Clue to Cancer") Study; EPIC, European Prospective Investigation into Cancer and Nutrition; FMC, Finnish Mobile Clinic Health Examination Survey; HIMS, Health in Men Study; HPFS, Health Professionals Follow-up Study; Janus part 1, Nordic Biological Specimen Biobank Working Group; Janus part 2, a second study using the Janus Serum Bank from Norway; JPHC, Japan Public Health Cohort; MCCS, Melbourne Collaborative Cohort Study; MDCS, Malmö Diet and Cancer Study; MEC, Multiethnic Cohort; N/A, data not available for this study; PCPT, Prostate Cancer Prevention Trial; PHS, Physicians Health Study; PLCO, Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial; SELECT, Selenium and Vitamin E Cancer Prevention Trial; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; 25(OH)D,25-hydroxyvitamin D ; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D.

Table 2. Characteristics of participants with prostate cancer[a]

Prospective studies	Time from blood collection to diagnosis (%)[a]			Age at diagnosis (%)[a]			Year of diagnosis (%)[a]			Disease stage, aggressiveness and grade (%)				
	<3 y	3-6 y	≥7 y	<60 y	60-69 y	≥70 y	Before 1990	1990-1994	1995-Onward	Advanced stage[b]	Unknown stage	Aggressive disease[b]	High grade[b]	Unknown grade
ARIC	3.6	13.1	83.3	9.7	48.9	41.4	1.1	12.4	86.4	16.6	21.6	10.9	-	15.0
ATBC	4.0	10.6	85.3	3.5	44.4	52.1	5.3	15.2	79.5	52.2	19.3	39.1	11.8	21.5
CLUE I	0.0	1.6	98.4	8.2	23.0	68.9	59.0	41.0	0.0	23.5	16.4	37.7	5.1	3.3
EPIC	33.1	50.9	16.0	17.2	62.6	20.3	0.0	0.8	99.2	26.2	28.7	21.3	10.4	16.1
ESTHER	24.1	38.4	37.5	2.3	44.0	53.7	0.0	0.0	100	-	100	11.6	-	100
FMC	6.2	16.8	77.0	10.6	34.2	55.3	87.0	13.0	0.0	-	100	42.2	-	100
HIMS	42.2	45.8	12.0	0.0	0.0	100	0.0	0.0	100	-	100	11.5	-	100
HPFS	23.9	42.8	33.3	12.5	37.5	50.0	0.0	6.0	94.0	4.3	8.6	7.7	8.6	11.2
Janus part 1	1.2	5.0	93.7	20.7	69.2	10.1	27.0	56.2	16.9	-	100	-	-	100
Janus part 2	2.0	4.2	93.7	40.6	32.1	27.3	0.7	6.0	93.4	27.7	29.4	22.8	-	100
JPHC	9.0	17.4	73.6	7.0	39.8	53.2	0.0	3.5	96.5	28.5	24.9	22.9	24.2	69.2
MCCS	15.4	22.7	61.9	16.6	47.8	35.6	0.0	6.2	93.8	11.4	7.1	15.0	13.6	6.4
MDCS	12.2	30.0	57.8	5.9	47.8	46.3	0.0	2.7	97.3	-	100	-	-	100
MEC	82.1	15.8	2.1	7.9	34.7	57.5	0.0	0.0	100	-	100	10.9	0.3	5.2
PCPT	11.5	27.7	60.9	1.5	50.5	48.0	0.0	0.3	99.7	1.7	2.5	0.8	4.9	2.5
PHS	7.6	17.0	75.5	11.8	50.7	37.5	25.6	59.9	14.6	13.7	3.8	24.6	10.1	3.6
PLCO	56.4	39.1	4.6	7.5	52.2	40.3	0.0	0.0	100	18.6	0.0	7.6	10.7	0.3
SELECT	39.9	58.3	1.8	10.4	56.3	33.3	0.0	0.0	100	1.0	1.4	1.2	7.0	13.6
SU.VI.MAX	7.1	20.7	72.3	26.6	66.9	6.5	0.0	0.0	100	-	100	2.2	9.9	6.5

[a] Percentages exclude cases with missing values. Percentages may not add up to 100 because of rounding. Stage and grade of disease are unavailable for some case patients; the percentages are shown in the "unknown stage" and "unknown grade" columns.

[b] A tumour was categorised as advanced stage if it was tumor-node-metastasis (TNM) stage T3 or T4 and/or N1+ and/or M1, stage III–IV, or the equivalent. Aggressive disease was defined as tumours with TNM stage T4 and/or N1+ and/or M1 and/or stage IV disease and/or death from prostate cancer. High grade was defined as Gleason sum 8 or higher, or equivalent (undifferentiated).

For expansion of study names see Table 1. Abbreviation: y, year.

Figure legends

Figure 1. Odds ratios (95% confidence intervals) for prostate cancer associated with study-specific fifths of season-standardised 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentration in prospective studies.

Estimates are from logistic regression conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height and body mass index. *P*trend was calculated by replacing the fifths of vitamin D with a continuous variable that was scored as 0, 0.25, 0.5, 0.75 and 1 in the conditional logistic regression model. Abbreviations: 80%le= 80 percentile; CI = confidence interval; Ptr = *P*trend.

Figure 2. Study-specific odds ratios (95% confidence intervals) for prostate cancer associated with an 80 percentile increase in season-standardised 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentration. A) Blood season-standardised 25-hydroxyvitamin D concentration; B) Blood season-standardised 1,25-dihydroxyvitamin D concentration.

Estimates are from logistic regression conditioned on the matching variables within each study and without mutual adjustment for the other analytes. Heterogeneity in linear trends between studies was tested by comparing the X^2 values for models with and without a (studies) x (linear trend) interaction term. For expansion of study names see Table 1.

Figure 3. Odds ratios (95% confidence intervals) for prostate cancer associated with a study-specific 80 percentile increase in season-standardised 25-hydroxyvitamin D in prospective studies for selected subgroups.
The odds ratios were conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height and body mass index. Tests for heterogeneity for the case-defined factors were obtained by fitting separate models for each subgroup and assuming independence of the ORs using a method analogous to a meta-analysis. Tests for heterogeneity for the other factors were assessed with a χ^2-test of interaction between the subgroup and continuous trend test variable. Note that the number of cases for each tumour subtype may be fewer than shown in the baseline tables since here the analysis for each subgroup of a case-defined factor is restricted to complete matched sets for each category of the factor in turn; some matched sets contain a mixture of subtypes and while controls are allocated case-defined characteristics in equal proportion to the cases, 25(OH)D may be unknown for some participants, leading to incomplete matched sets.

Stage (early, T1 and/or stage I; other localized, T2/N0/M0 and/or stage II, and advanced, T3-T4/N1/M1 and/or stage III-IV), grade (low-intermediate, Gleason sum was < 8 or equivalent; high, Gleason sum was ≥ 8 or equivalent, and aggressive (T4/N1/M1 and/or stage IV and/or prostate cancer death). White ethnicity (89.4% yes, 10.6% no).

Figure 4. Odds ratios (95% confidence intervals) for prostate cancer associated with a study-specific 80 percentile increase in 25-hydroxyvitamin D (A) and 1,25-dihydroxyvitamind D (B) concentration by season.

The odds ratios were conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height and body mass index. Tests for heterogeneity were assessed with a χ^2-test of interaction between the subgroup and continuous trend test variable. A) Blood 25-hydroxyvitamin D concentration; B) Blood 1,25-dihydroxyvitamin D concentration

Figure 5. Geometric mean concentrations (95% confidence intervals) of season-standardised 25-hydroxyvitamin D (nmol/L) for controls from all studies by various factors, adjusted for study and age at blood collection.

Means are scaled to, and depicted as a proportion of, the overall geometric mean concentration (dotted line). P values are for tests of heterogeneity and, where applicable in parentheses, trend.

Figure 1

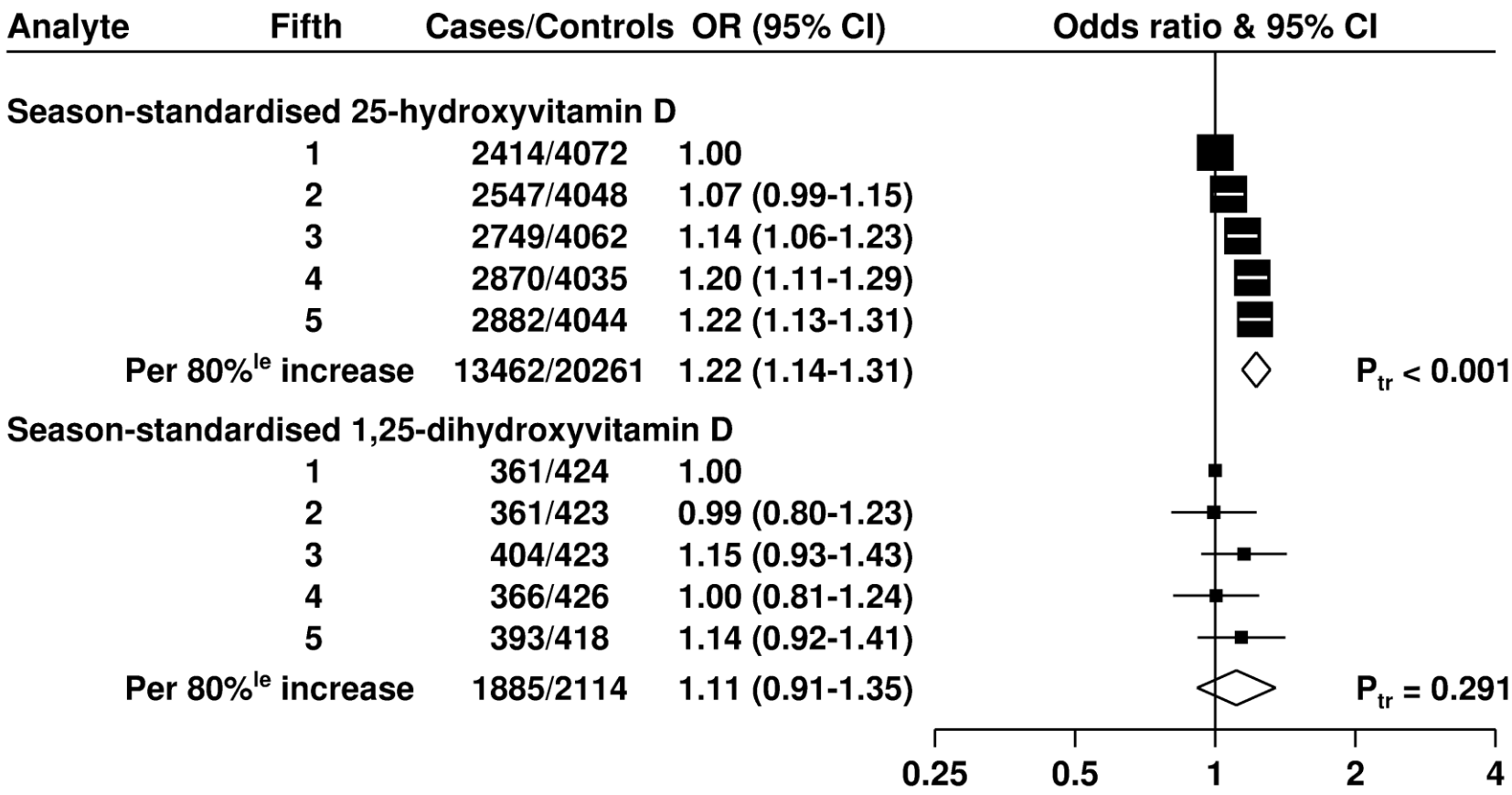
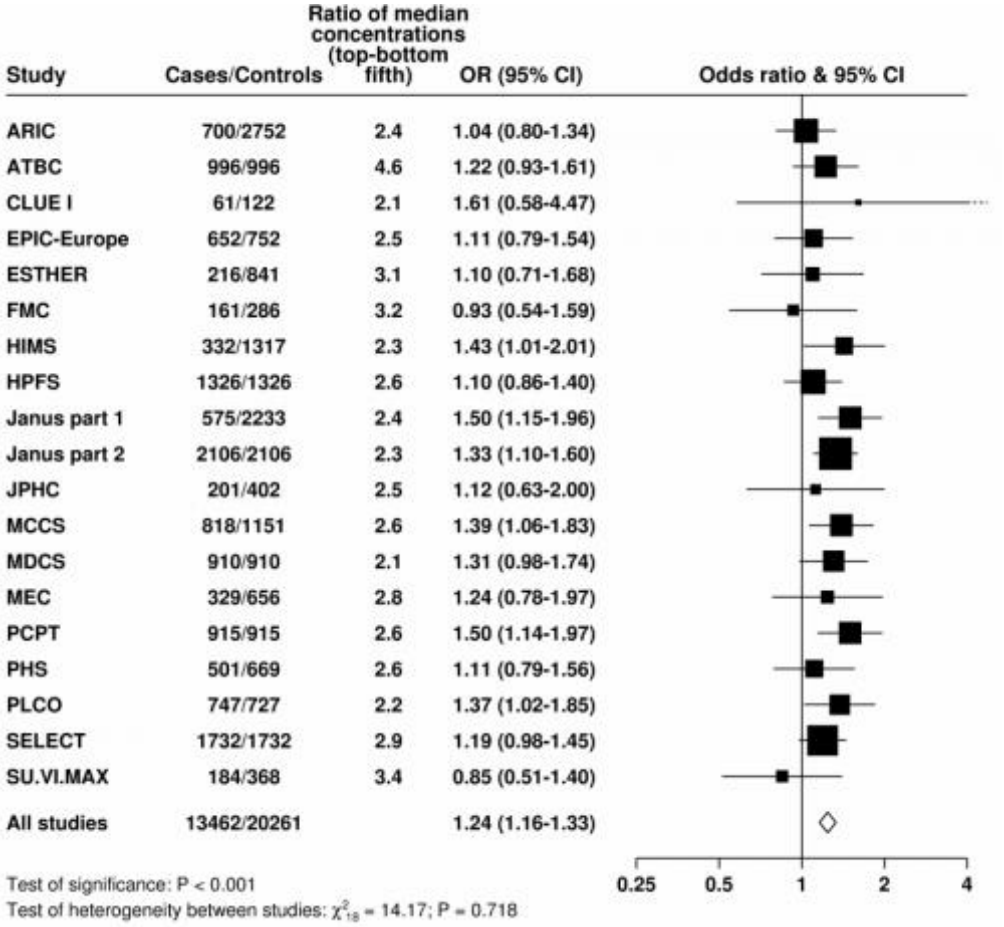

Analyte
Fifth
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
Season-standardised 25-hydroxyvitamin D
1 2414/4072 1.00
2 2547/4048 1.07 (0.99-1.15)
3 2749/4062 1.14 (1.06-1.23)
4 2870/4035 1.20 (1.11-1.29)
5 2882/4044 1.22 (1.13-1.31)
Per 80%le increase 13462/20261 1.22 (1.14-1.31)
Ptr < 0.001
Season-standardised 1,25-dihydroxyvitamin D
1 361/424 1.00
2 361/423 0.99 (0.80-1.23)
3 404/423 1.15 (0.93-1.43)
4 366/426 1.00 (0.81-1.24)
5 393/418 1.14 (0.92-1.41)
Per 80%le increase 1885/2114 1.11 (0.91-1.35)
Ptr = 0.291
0.25
0.5
1
2
4

Figure 2

A


Study
Cases/Controls
Ratio of median concentrations (top-bottom fifth)
OR (95% CI)
Odds ratio & 95% CI
ARIC 700/2752 2.4 1.04 (0.80-1.34)
ATBC 996/996 4.6 1.22 (0.93-1.61)
CLUE I 61/122 2.1 1.61 (0.58-4.47)
EPIC-Europe 652/752 2.5 1.11 (0.79-1.54)
ESTHER 216/841 3.1 1.10 (0.71-1.68)
FMC 161/286 3.2 0.93 (0.54-1.59)
HIMS 332/1317 2.3 1.43 (1.01-2.01)
HPFS 1326/1326 2.6 1.10 (0.86-1.40)
Janus part 1 575/2233 2.4 1.50 (1.15-1.96)
Janus part 2 2106/2106 2.3 1.33 (1.10-1.60)
JPHC 201/402 2.5 1.12 (0.63-2.00)
MCCS 818/1151 2.6 1.39 (1.06-1.83)
MDCS 910/910 2.1 1.31 (0.98-1.74)
MEC 329/656 2.8 1.24 (0.78-1.97)
PCPT 915/915 2.6 1.50 (1.14-1.97)
PHS 501/669 2.6 1.11 (0.79-1.56)
PLCO 747/727 2.2 1.37 (1.02-1.85)
SELECT 1732/1732 2.9 1.19 (0.98-1.45)
SU.VI.MAX 184/368 3.4 0.85 (0.51-1.40)
All studies 13462/20261 1.24 (1.16-1.33)
0.25
0.5
1
2
4
Test of significance: P < 0.001
Test of heterogeneity between studies: χ^2_{18} = 14.17; P = 0.718

B

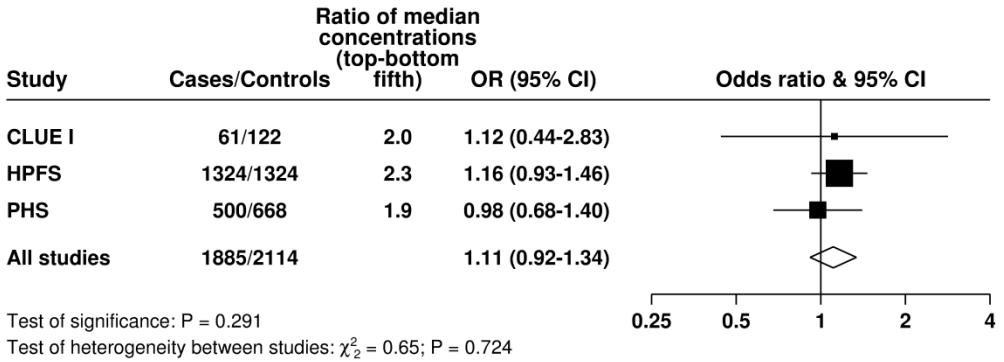

Study
Cases/Controls
Ratio of median concentrations (top-bottom fifth)
OR (95% CI)
Odds ratio & 95% CI
CLUE I 61/122 2.0 1.12 (0.44-2.83)
HPFS 1324/1324 2.3 1.16 (0.93-1.46)
PHS 500/668 1.9 0.98 (0.68-1.40)
All studies 1885/2114 1.11 (0.92-1.34)
0.25
0.5
1
2
4
Test of significance: P = 0.291
Test of heterogeneity between studies: χ^2_2 = 0.65; P = 0.724

Figure 3

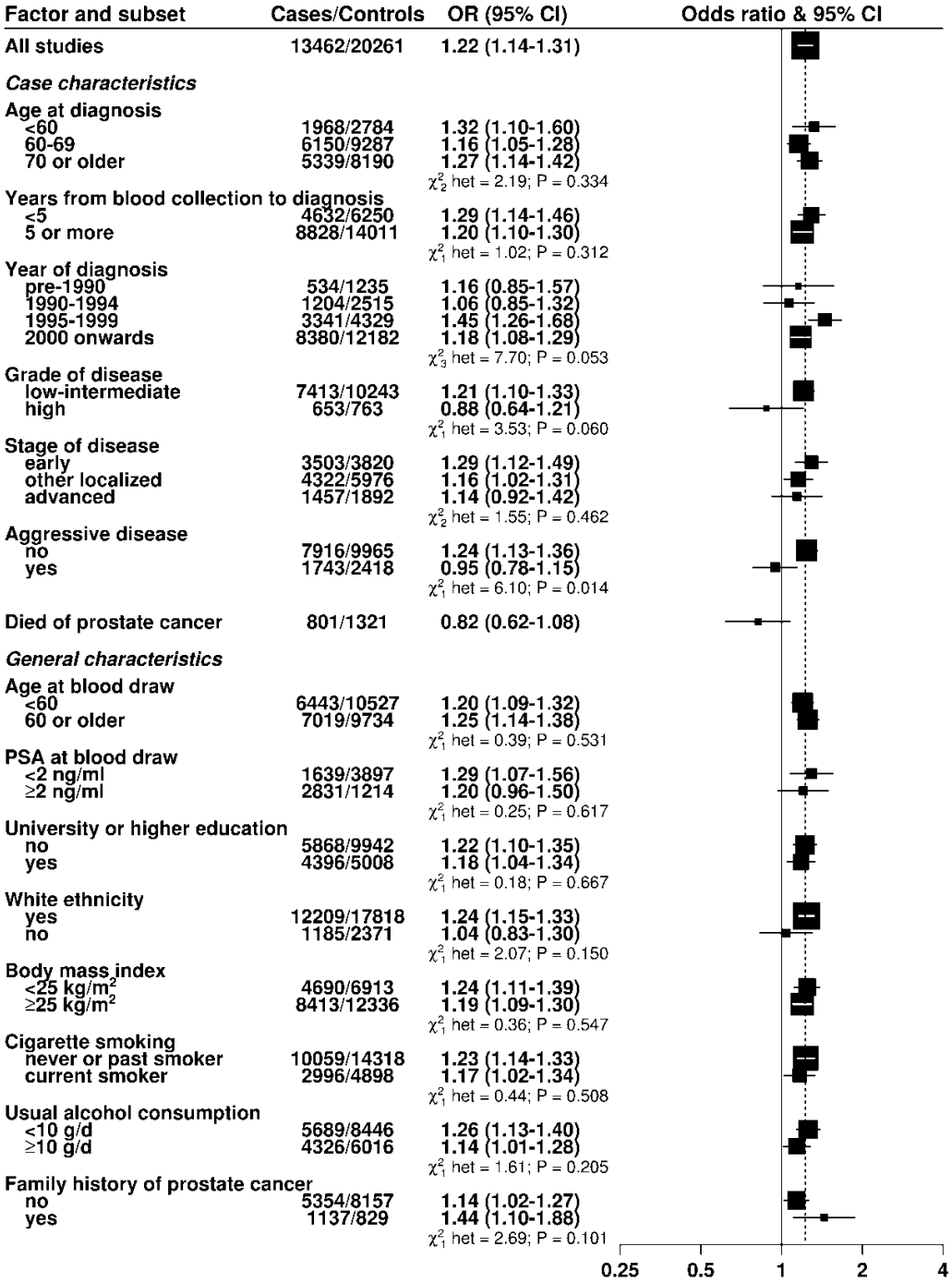
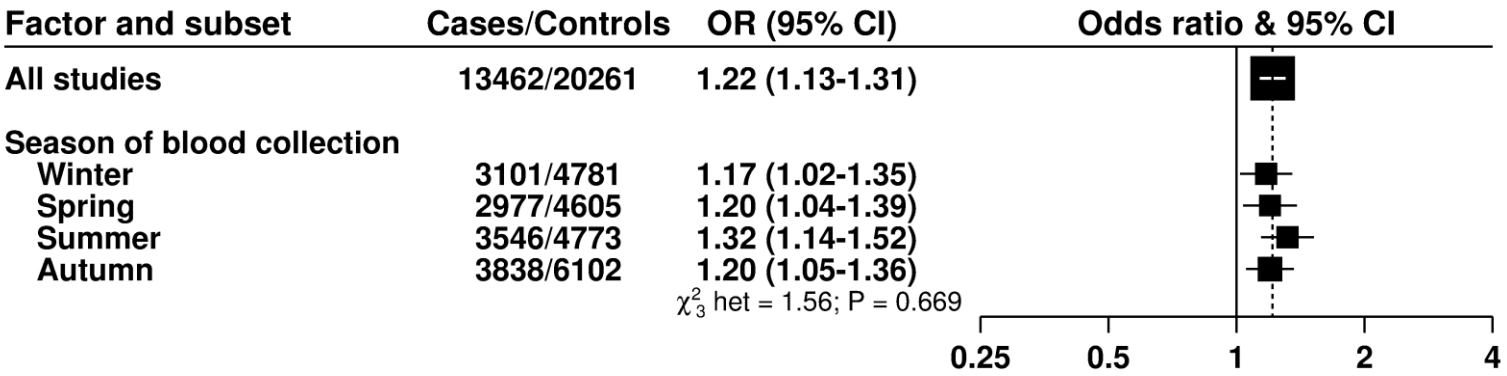

Factor and subset
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
All studies 13462/20261 1.22 (1.14-1.31)
Case characteristics
Age at diagnosis
<60 1968/2784 1.32 (1.10-1.60)
60-69 6150/9287 1.16 (1.05-1.28)
70 or older 5339/8190 1.27 (1.14-1.42)
χ²₂ het = 2.19; P = 0.334
Years from blood collection to diagnosis
<5 4632/6250 1.29 (1.14-1.46)
5 or more 8828/14011 1.20 (1.10-1.30)
χ²₁ het = 1.02; P = 0.312
Year of diagnosis
pre-1990 534/1235 1.16 (0.85-1.57)
1990-1994 1204/2515 1.06 (0.85-1.32)
1995-1999 3341/4329 1.45 (1.26-1.68)
2000 onwards 8380/12182 1.18 (1.08-1.29)
χ²₃ het = 7.70; P = 0.053
Grade of disease
low-intermediate 7413/10243 1.21 (1.10-1.33)
high 653/763 0.88 (0.64-1.21)
χ²₁ het = 3.53; P = 0.060
Stage of disease
early 3503/3820 1.29 (1.12-1.49)
other localized 4322/5976 1.16 (1.02-1.31)
advanced 1457/1892 1.14 (0.92-1.42)
χ²₂ het = 1.55; P = 0.462
Aggressive disease
no 7916/9965 1.24 (1.13-1.36)
yes 1743/2418 0.95 (0.78-1.15)
χ²₁ het = 6.10; P = 0.014
Died of prostate cancer 801/1321 0.82 (0.62-1.08)
General characteristics
Age at blood draw
<60 6443/10527 1.20 (1.09-1.32)
60 or older 7019/9734 1.25 (1.14-1.38)
χ²₁ het = 0.39; P = 0.531
PSA at blood draw
<2 ng/ml 1639/3897 1.29 (1.07-1.56)
≥2 ng/ml 2831/1214 1.20 (0.96-1.50)
χ²₁ het = 0.25; P = 0.617
University or higher education
no 5868/9942 1.22 (1.10-1.35)
yes 4396/5008 1.18 (1.04-1.34)
χ²₁ het = 0.18; P = 0.667
White ethnicity
yes 12209/17818 1.24 (1.15-1.33)
no 1185/2371 1.04 (0.83-1.30)
χ²₁ het = 2.07; P = 0.150
Body mass index
<25 kg/m² 4690/6913 1.24 (1.11-1.39)
≥25 kg/m² 8413/12336 1.19 (1.09-1.30)
χ²₁ het = 0.36; P = 0.547
Cigarette smoking
never or past smoker 10059/14318 1.23 (1.14-1.33)
current smoker 2996/4898 1.17 (1.02-1.34)
χ²₁ het = 0.44; P = 0.508
Usual alcohol consumption
<10 g/d 5689/8446 1.26 (1.13-1.40)
≥10 g/d 4326/6016 1.14 (1.01-1.28)
χ²₁ het = 1.61; P = 0.205
Family history of prostate cancer
no 5354/8157 1.14 (1.02-1.27)
yes 1137/829 1.44 (1.10-1.88)
χ²₁ het = 2.69; P = 0.101
0.25
0.5
1
2
4

Figure 4

A


Factor and subset
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
All studies
13462/20261
1.22 (1.13-1.31)
Season of blood collection
Winter
3101/4781
1.17 (1.02-1.35)
Spring
2977/4605
1.20 (1.04-1.39)
Summer
3546/4773
1.32 (1.14-1.52)
Autumn
3838/6102
1.20 (1.05-1.36)
χ^2_3 het = 1.56; P = 0.669
0.25
0.5
1
2
4

B

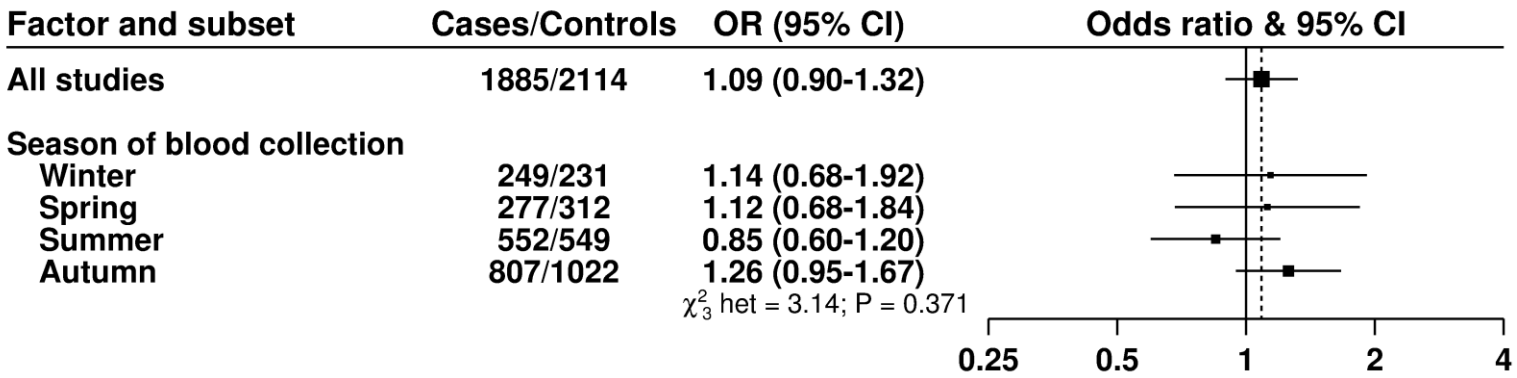
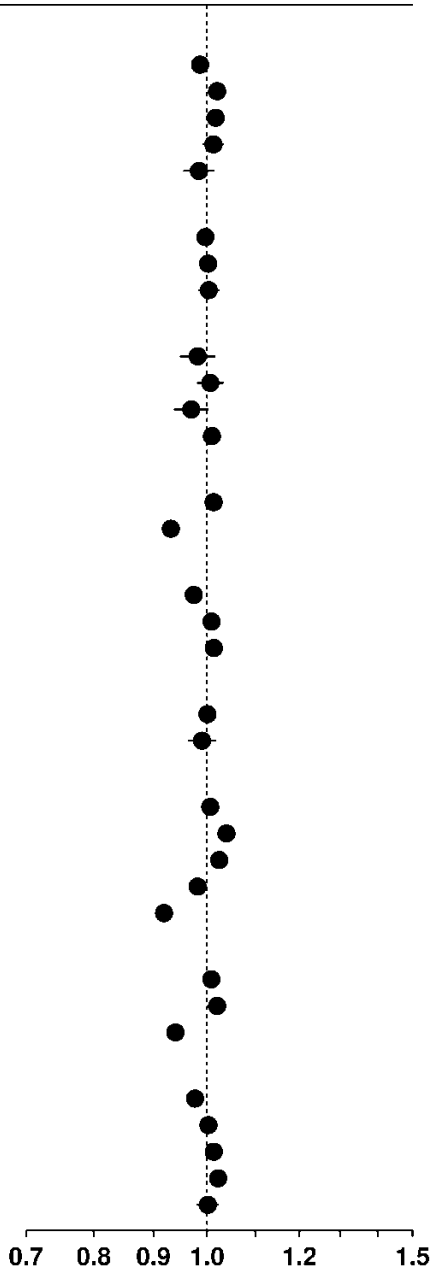

Factor and subset
Cases/Controls
OR (95% CI)
Odds ratio & 95% CI
All studies
1885/2114
1.09 (0.90-1.32)
Season of blood collection
Winter
249/231
1.14 (0.68-1.92)
Spring
277/312
1.12 (0.68-1.84)
Summer
552/549
0.85 (0.60-1.20)
Autumn
807/1022
1.26 (0.95-1.67)
χ^2_3 het = 3.14; P = 0.371
0.25
0.5
1
2
4

Figure 5

Factor and subset	n	Mean* (95% CI)	P values#	Relative mean+ & 95% CI
Age at blood collection@				
under 60	10966	56.8 (56.3-57.2)	<0.001	
60-64	4441	58.7 (58.0-59.4)	(0.009)	
65-69	3245	58.5 (57.7-59.4)		
70-74	1871	58.3 (57.1-59.5)		
75 and over	1178	56.6 (54.9-58.4)		
Time of blood collection@				
0000-0959	4285	57.4 (56.6-58.2)	0.848	
1000-1259	2740	57.7 (56.7-58.7)		
1300-2359	1950	57.7 (56.5-59.0)		
Time since last meal (hours)				
under 3	794	56.5 (54.5-58.5)	0.145	
3-5	1446	57.9 (56.4-59.5)	(0.350)	
6-11	785	55.8 (53.9-57.7)		
12 or more	2989	58.1 (57.0-59.2)		
Married or cohabiting				
yes	12268	58.3 (57.9-58.7)	<0.001	
no	2257	53.6 (52.7-54.5)		
Educational attainment@				
below secondary/HS	3739	56.1 (55.2-56.9)	0.001	
secondary/HS/college	6282	58.1 (57.4-58.7)		
university	3026	58.3 (57.4-59.2)		
Father or brother with prostate cancer				
no	9711	57.6 (57.1-58.1)	0.469	
yes	962	57.0 (55.4-58.6)		
Body mass index (kg/m^2)@				
<22.5	2385	57.9 (57.0-58.9)	<0.001	
22.5-24.9	5001	59.8 (59.1-60.5)	(<0.001)	
25.0-27.4	6012	58.9 (58.4-59.5)		
27.5-29.9	3815	56.5 (55.8-57.2)		
≥30.0	3331	52.9 (52.1-53.6)		
Cigarette smoking@				
never	5887	58.0 (57.5-58.6)	<0.001	
previous	8172	58.7 (58.2-59.2)		
current	3453	54.1 (53.3-54.8)		
Usual alcohol consumption@				
none	4668	56.2 (55.5-56.9)	<0.001	
1-9 g/d	4350	57.7 (57.0-58.4)	(<0.001)	
10-19 g/d	2852	58.3 (57.4-59.2)		
20-39 g/d	2555	58.8 (57.9-59.8)		
≥40 g/d	1505	57.6 (56.4-58.9)		



* means are scaled to the overall geometric mean concentration
# P values for tests of heterogeneity and, where applicable and in parenthesis, trend
+ values are depicted as a proportion of the overall geometric mean concentration (dotted line)
@ P<0.05 for test of interaction with study

Supplementary methods

Data collection

The EHNBPCCG is described in detail elsewhere [1-7]. Published and unpublished studies were eligible for the current collaborative individual participant meta-analysis if they had data on pre-diagnostic circulating concentrations of 25(OH)D or $1,25(OH)_2D$ and incident prostate cancers. Studies were identified through searches using the terms "vitamin D", "25-hydroxyvitamin D", "1,25 dihydroxyvitamin D", and "prostate cancer" on computerized bibliographic systems, including PubMed, Web of Science, Cochrane Library, and CancerLit, through the reference lists of publications identified in this search, and through correspondence with study investigators.

Individual participant data on circulating 25(OH)D for 13,462 men with prostate cancer and 20,261 control participants were available from 19 prospective cohort studies by the date of dataset closure (May 2018): the Atherosclerosis Risk in Communities (ARIC) Study (unpublished, study described in [8], Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) [9]; Campaign Against Cancer and Stroke ("Give Us a Clue to Cancer") Study (CLUE) I [10]; Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten THerapie chronischer ERkrankungen in der älteren Bevölkerung (ESTHER) [11]; European Prospective Investigation into Cancer and Nutrition (EPIC) [12]; Finnish Mobile Clinic Health Examination Survey (FMC) (unpublished, study described in [13]); Health Professionals Follow-up Study (HPFS) [14-16]; Health In Men Study (HIMS) [17]; the Janus study that formed part of the of the Nordic Biological Specimen Biobank Working Group (Janus part 1) [18]; a second study using the Janus Serum Bank (Janus part 2) [19]; Japan Public Health Center–based Prospective (JPHC) Study [20]; Melbourne Collaborative Cohort Study (MCCS) (unpublished, study described in [21]); Malmö Diet and Cancer Study (MDCS) [22]; Multiethnic Cohort (MEC) [23]; Prostate Cancer Prevention Trial (PCPT) [24]; Physicians' Health Study (PHS) [25-27]; Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) [28]; Selenium and Vitamin E Cancer Prevention Trial (SELECT) [29]; and the SUpplémentation en VItamines et Minéraux AntioXydants (SU.VI.MAX) trial [30]. Data on 25(OH)D from seven relatively small studies (with a combined total of 1,047 cases) were not available for pooling: Helsinki Heart Study (HHS) [18, 31], Japan-Hawaii Cancer Study (JHCS) [32], Kaiser Permanente Medical Care Programme (KPMCP) [33, 34], The Osteoporotic Fractures in Men (MrOS) Study [35], Nutritional Prevention of Cancer (*NPC*) Trial [36], Northern Sweden Health and Disease Cohort (NSHDC) [18], and the Tromsø cohort study [37].

Individual participant data on circulating $1,25(OH)_2D$ for 1885 men with prostate cancer and 2114 control participants were available from three studies (CLUE I, HPFS and PHS) [10, 14, 25], but were not available for KPMCP [33, 34] or JHCS [32].

Individual participant data were requested on circulating 25(OH)D and 1,25(OH)2D, date, age and fasting status at sample collection, marital status, ethnicity, educational attainment, family history of prostate cancer, height, weight, waist and hip circumference, smoking status, alcohol intake, and vital status. Each study also provided data on prostate cancer stage and grade and death, if available, and the data were harmonized in a central database.

Study designs and data processing

Most of the studies were case-control studies nested within prospective cohort studies, with some variation between the studies in the case mix, related for example to the prevalence of Prostate Specific Antigen (PSA)-testing within that population during follow-up. Four studies (ARIC, ESTHER, HIMS, and MCCS) provided cohort data; therefore cases and controls were matched at the pooling center (University of Oxford) (for details about the matching criteria please see **Supplementary Table 1**). Two studies (PCPT and PLCO) were observational investigations based within randomized controlled trials that included organized screening for prostate cancer [24, 28]. For both these studies, men with a raised prostate specific antigen (PSA) or abnormal digital rectal examination at recruitment were excluded, and the majority of cases were detected either through subsequent PSA-screening (PLCO and PCPT) or by end-of-study biopsy (PCPT). Data on circulating 25(OH)D and $1,25(OH)_2D$ for 1,424 prostate cancer cases and 1,440 control participants from a cross-sectional study within The Prostate Testing for Cancer and Treatment (*ProtecT*) trial were also available for analysis. In this study, all men with a PSA≥3 ng/mL at recruitment were offered diagnostic biopsy and those diagnosed at this time were included as cases for the observational study [38, 39]. Data on the control participants are included in cross-sectional analyses of vitamin D concentrations in relation to participant characteristics, but because cases were diagnosed at the start of the study rather than during follow-up data, these data were not included in the main risk analyses.

Details of the recruitment of participants, informed consent and ethics approvals are provided in the original publications [8-12, 14-20, 22-30, 38, 39].

Statistical analyses

The methods of analysis were similar to those described previously by this collaborative group [3, 5]. 25(OH)D and 1,25$(OH)_2$D concentrations were used as provided by the authors and were log-transformed to approximate a normal distribution for parametric analyses. To allow for the influence of month of blood draw on circulating concentrations, a regression model of log-transformed vitamin D concentration by month of blood collection (as a categorical variable) was fitted for each study; the "season-standardized" concentrations of 25(OH)D and 1,25$(OH)_2$D were then calculated by subtracting the residuals from each regression model from the study-specific mean log vitamin D concentration, and then exponentiating these values. Thus, the "season-standardized" values represent vitamin D concentration 'corrected' for month of blood collection. All results are presented by season-standardized vitamin D, unless otherwise specified.

The main method of analysis was logistic regression conditioned on the matching variables within each study. Men were categorized into fifths of the distribution of 25(OH)D and 1,25$(OH)_2$D, with cut-points defined by the study-specific quintiles of the distribution within control participants, to allow for any systematic differences between the studies in assay methods and blood sample types [40]. In order to provide a summary measure of the OR, a linear trend was calculated by replacing the categorical variable representing the fifths of each analyte with a continuous variable that was scored as 0, 0.25, 0.5, 0.75, and 1; because the mid-points of the lowest and highest fifths are the 10^{th} and 90^{th} percentiles of the study-specific vitamin D concentration, a unit increase in this variable can be taken to represent an 80 percentile increase in the study-specific concentration of vitamin D. To assess the risk for prostate cancer risk in men with very low vitamin D concentrations, season-standardized 25(OH)D was also categorized into study-specific tenths.

To examine the effects of potential confounders (other than the matching criteria, which were taken into account in the study design and matched analyses), conditional logistic regression analyses were performed that included the following covariates: age at blood collection (continuous), body mass index (BMI, continuous), height (continuous), marital status (married or cohabiting, not married or cohabiting, or not known), educational status (did not graduate from high school/secondary school/college, high school/secondary school/college graduates, university graduates, or not known) and cigarette smoking (never smoker, past smoker, current, or not known), all of which were associated with prostate cancer risk in these analyses.

In a sensitivity analysis, conditional logistic regression models were also fitted using quintile cut-points defined by the overall distribution among the control participants in all studies combined; this approach maximizes the ability to examine associations across the full distribution of biomarker concentration across all

studies but assumes that the differences in absolute values between studies are due to true population differences, rather than due to assay differences between the studies. The analyses were also repeated using predefined categories for concentrations of 25(OH)D of <30, 30-<50, 50-<75 and ≥75 nmol/L, in order to investigate risks associated with very low (deficiency), low (insufficiency), moderate (sufficiency) and high circulating concentrations of vitamin D based on the Institute of Medicine (IoM) recommendations [41]. We also assessed whether circulating concentrations of vitamin D were related to death from prostate cancer.

For each analyte, heterogeneity in linear trends between studies was assessed by comparing the χ^2 values for models with and without a (study) x (linear trend) interaction term. To test whether the estimates for each analyte varied according to case characteristics, ORs were estimated within a series of subsets for the following characteristics: age at diagnosis, years from blood collection to diagnosis, year of diagnosis, stage of disease, aggressive disease, and grade of disease. Controls in each matched set were assigned the value of their matched case for the case-defined factors (e.g. age at diagnosis, years from blood collection to diagnosis). For the multi-matched sets in PLCO in which the case characteristics varied (e.g. some low-intermediate grade, some high grade), controls were randomly allocated to cases in the same proportions. Tests for heterogeneity for the case-defined factors (were obtained by fitting separate models for each subgroup and assuming independence of the ORs using a method analogous to a meta-analysis. Subgroup analyses were also conducted by age at blood draw, PSA at blood draw, university or higher education, BMI, cigarette smoking, alcohol consumption, season of blood draw, ethnicity and family history of prostate cancer. Tests for heterogeneity for these factors were assessed with a χ^2-test of interaction between subgroup and the continuous trend test variable.

In order to assess potential effect modification with different biomarkers, a χ^2-test of interaction was used to determine whether risks by study-specific thirds of 25(OH)D varied according to study-specific thirds of $1,25(OH)_2D$ (and vice versa), and according to study-specific thirds of circulating concentrations of insulin-like growth factor-I (IGF)-I, IGF binding protein-3 (IGFBP3), testosterone, free testosterone, sex hormone-binding globulin (SHBG) and prostate-specific antigen (PSA), where these data were available.

To explore the relationships between analytes, partial correlation coefficients between season-standardized 25(OH)D and $1,25(OH)_2D$ and other selected circulating biomarkers were calculated using standardized log-transformed concentrations among controls from each study, adjusting for age at blood collection and, in a second analysis, also for BMI. Standardization (by subtracting the mean log concentration and

dividing by the standard deviation of the log concentration) was performed to minimize for any systematic differences in the biomarker concentration between studies owing to differences in the assays.

The cross-sectional associations of 25(OH)D and $1,25(OH)_2D$ with participant characteristics (among the controls) were examined using analyses of variance to calculate geometric mean concentrations and 95% confidence intervals (CIs), adjusting for study and age at blood collection, as appropriate. F tests were used to test for heterogeneity in the geometric mean analyte concentrations between the categories, and where appropriate, to test for trends across the categories, with the ordered categories scored from 1 to the maximum number of categories.

All tests of statistical significance were two-sided, and statistical significance was set at the 5% level. All statistical tests were carried out with *Stata Statistical Software, Release 14* (StataCorp, LP, College Station, Texas).

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References for the supplementary methods

1. Roddam AW, Allen NE, Appleby P, et al. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. J Natl Cancer Inst 2008;100(3):170-83.

2. Roddam AW, Allen NE, Appleby P, et al. Insulin-like growth factors, their binding proteins, and prostate cancer risk: analysis of individual patient data from 12 prospective studies. Ann Intern Med 2008;149(7):461-71, W83-8.

3. Travis RC, Appleby PN, Martin RM, et al. A meta-analysis of individual participant data reveals an association between circulating levels of IGF-I and prostate cancer risk. Cancer Res 2016; 10.1158/0008-5472.CAN-15-1551.

4. Crowe FL, Appleby PN, Travis RC, et al. Circulating fatty acids and prostate cancer risk: individual participant meta-analysis of prospective studies. J Natl Cancer Inst 2014;106(9).

5. Allen NE, Travis RC, Appleby PN, et al. Selenium and Prostate Cancer: Analysis of Individual Participant Data From Fifteen Prospective Studies. J Natl Cancer Inst 2016;108(11).

6. Key TJ, Appleby PN, Allen NE, et al. Plasma carotenoids, retinol, and tocopherols and the risk of prostate cancer in the European Prospective Investigation into Cancer and Nutrition study. Am J Clin Nutr 2007;86(3):672-81.

7. Price AJ, Travis RC, Appleby PN, et al. Circulating Folate and Vitamin B and Risk of Prostate Cancer: A Collaborative Analysis of Individual Participant Data from Six Cohorts Including 6875 Cases and 8104 Controls. Eur Urol 2016; 10.1016/j.eururo.2016.03.029.

8. Joshu CE, Barber JR, Coresh J, et al. Enhancing the Infrastructure of the Atherosclerosis Risk in Communities (ARIC) Study for Cancer Epidemiology Research: ARIC Cancer. Cancer Epidemiol Biomarkers Prev 2018;27(3):295-305.

9. Albanes D, Mondul AM, Yu K, et al. Serum 25-hydroxy vitamin D and prostate cancer risk in a large nested case-control study. Cancer Epidemiol Biomarkers Prev 2011;20(9):1850-60.

10. Braun MM, Helzlsouer KJ, Hollis BW, et al. Prostate cancer and prediagnostic levels of serum vitamin D metabolites (Maryland, United States). Cancer Causes Control 1995;6(3):235-9.

11. Ordonez-Mena JM, Schottker B, Haug U, et al. Serum 25-hydroxyvitamin d and cancer risk in older adults: results from a large German prospective cohort study. Cancer Epidemiol Biomarkers Prev 2013;22(5):905-16.

12. Travis RC, Crowe FL, Allen NE, et al. Serum vitamin D and risk of prostate cancer in a case-control analysis nested within the European Prospective Investigation into Cancer and Nutrition (EPIC). Am J Epidemiol 2009;169(10):1223-32.

13. Knekt P, Laaksonen M, Mattila C, et al. Serum vitamin D and subsequent occurrence of type 2 diabetes. Epidemiology 2008;19(5):666-671.

14. Platz EA, Leitzmann MF, Hollis BW, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control 2004;15(3):255-65.

15. Mikhak B, Hunter DJ, Spiegelman D, et al. Vitamin D receptor (VDR) gene polymorphisms and haplotypes, interactions with plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, and prostate cancer risk. Prostate 2007;67(9):911-23.

16. Shui IM, Mucci LA, Kraft P, et al. Vitamin D-related genetic variation, plasma vitamin D, and risk of lethal prostate cancer: a prospective nested case-control study. J Natl Cancer Inst 2012;104(9):690-9.

17. Wong YY, Hyde Z, McCaul KA, et al. In older men, lower plasma 25-hydroxyvitamin D is associated with reduced incidence of prostate, but not colorectal or lung cancer. PLoS One 2014;9(6):e99954.

18. Tuohimaa P, Tenkanen L, Ahonen M, et al. Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer 2004;108(1):104-8.

19. Meyer HE, Robsahm TE, Bjorge T, et al. Vitamin D, season, and risk of prostate cancer: a nested case-control study within Norwegian health studies. Am J Clin Nutr 2013;97(1):147-54.

20. Sawada N, Inoue M, Iwasaki M, et al. Plasma 25-hydroxy vitamin D and subsequent prostate cancer risk in a nested Case-Control study in Japan: The JPHC study. Eur J Clin Nutr 2017;71(1):132-136.

21. Milne RL, Fletcher AS, MacInnis RJ, et al. Cohort Profile: The Melbourne Collaborative Cohort Study (Health 2020). Int J Epidemiol 2017;46(6):1757-1757i.

22. Brandstedt J, Almquist M, Manjer J, et al. Vitamin D, PTH, and calcium and the risk of prostate cancer: a prospective nested case-control study. Cancer Causes Control 2012;23(8):1377-85.

23. Park SY, Cooney RV, Wilkens LR, et al. Plasma 25-hydroxyvitamin D and prostate cancer risk: the multiethnic cohort. Eur J Cancer 2010;46(5):932-6.

24. Schenk JM, Till CA, Tangen CM, et al. Serum 25-hydroxyvitamin d concentrations and risk of prostate cancer: results from the Prostate Cancer Prevention Trial. Cancer Epidemiol Biomarkers Prev 2014;23(8):1484-93.

25. Gann PH, Ma J, Hennekens CH, et al. Circulating vitamin D metabolites in relation to subsequent development of prostate cancer. Cancer Epidemiol Biomarkers Prev 1996;5(2):121-6.

26. Li H, Stampfer MJ, Hollis JB, et al. A prospective study of plasma vitamin D metabolites, vitamin D receptor polymorphisms, and prostate cancer. PLoS Med 2007;4(3):e103.

27. Ma J, Stampfer MJ, Gann PH, et al. Vitamin D receptor polymorphisms, circulating vitamin D metabolites, and risk of prostate cancer in United States physicians. Cancer Epidemiol Biomarkers Prev 1998;7(5):385-90.

28. Ahn J, Albanes D, Berndt SI, et al. Vitamin D-related genes, serum vitamin D concentrations and prostate cancer risk. Carcinogenesis 2009;30(5):769-76.

29. Kristal AR, Till C, Song X, et al. Plasma vitamin D and prostate cancer risk: results from the Selenium and Vitamin E Cancer Prevention Trial. Cancer Epidemiol Biomarkers Prev 2014;23(8):1494-504.

30. Deschasaux M, Souberbielle JC, Latino-Martel P, et al. A prospective study of plasma 25-hydroxyvitamin D concentration and prostate cancer risk. Br J Nutr 2016;115(2):305-14.

31. Ahonen MH, Tenkanen L, Teppo L, et al. Prostate cancer risk and prediagnostic serum 25-hydroxyvitamin D levels (Finland). Cancer Causes Control 2000;11(9):847-52.

32. Nomura AM, Stemmermann GN, Lee J, et al. Serum vitamin D metabolite levels and the subsequent development of prostate cancer (Hawaii, United States). Cancer Causes Control 1998;9(4):425-32.

33. Corder EH, Friedman GD, Vogelman JH, et al. Seasonal variation in vitamin D, vitamin D-binding protein, and dehydroepiandrosterone: risk of prostate cancer in black and white men. Cancer Epidemiol Biomarkers Prev 1995;4(6):655-9.

34. Corder EH, Guess HA, Hulka BS, et al. Vitamin D and prostate cancer: a prediagnostic study with stored sera. Cancer Epidemiol Biomarkers Prev 1993;2(5):467-72.

35. Barnett CM, Nielson CM, Shannon J, et al. Serum 25-OH vitamin D levels and risk of developing prostate cancer in older men. Cancer Causes Control 2010;21(8):1297-303.

36. Jacobs ET, Giuliano AR, Martinez ME, et al. Plasma levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and the risk of prostate cancer. J Steroid Biochem Mol Biol 2004;89-90(1-5):533-7.

37. Ordonez-Mena JM, Schottker B, Fedirko V, et al. Pre-diagnostic vitamin D concentrations and cancer risks in older individuals: an analysis of cohorts participating in the CHANCES consortium. Eur J Epidemiol 2016;31(3):311-23.

38. Gilbert R, Metcalfe C, Fraser WD, et al. Associations of circulating retinol, vitamin E, and 1,25-dihydroxyvitamin D with prostate cancer diagnosis, stage, and grade. Cancer Causes Control 2012;23(11):1865-73.

39. Gilbert R, Metcalfe C, Fraser WD, et al. Associations of circulating 25-hydroxyvitamin D with prostate cancer diagnosis, stage and grade. Int J Cancer 2012;131(5):1187-96.

40. Key TJ, Appleby PN, Allen NE, et al. Pooling biomarker data from different studies of disease risk, with a focus on endogenous hormones. Cancer Epidemiol Biomarkers Prev 2010;19(4):960-5.

41. In: Ross AC, Taylor CL, Yaktine AL, et al., (eds). Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC); 2011.

Supplementary tables and figures

Supplementary Table 1. Study characteristics

Study (First author, year)	Sample population	Location	Recruitment period	Prostate cancer ascertainment method	Nested case-control study characteristics	
					Ratio of case patients to control participants	Matching criteria and comments
ARIC (Unpublished)[d]	Population-based cohort study	USA	1987-1989	Cancer registry linkage supplemented with medical records; death certificates	1:1-4 [b]	Age at and date of blood collection (each ± 24 months), ethnicity, and requiring that controls had a vitamin D measurement if the matched case had one.
ATBC (Albanes *et al.*, 2011)	Randomized trial of α-tocopherol and β-carotene among smokers	Finland	1985-1988	Cancer registry linkage	1:1	Age at randomization (±1 year) and date of baseline blood collection (±30 days)
CLUE 1 (Braun *et al.*, 1995)	Population-based cohort study	USA	1974	Cancer registry linkage	1:2	Age (±1 year)
EPIC (Travis *et al.*, 2009)	Population-based cohort study	Europe	1991-2001	Cancer registry linkage; health insurance record linkage; Self-report with medical record review	1:1 except for the Umeå center which was 1:2	Study center, age enrolment (±6 months), time of blood draw (±1 hour), time between blood draw and last consumption of food/drink (<3 ,3-6, >6 hours; for Umeå <4, 4-8, >8 hours)
ESTHER (Ordonez-Mena *et al.*, 2013)	Population-based cohort study	Germany	2000-2002	Active follow-up and record linkage with national and regional cancer registries	1:4 [b]	Age at blood collection (±12 months), month of the year (but not necessarily year) of blood collection (± 1 month), fasting status, ethnicity, and family history of prostate cancer (where known for the case).
FMC (unpublished)[a]	Population-based cohort study	Finland	1968-1972	Cancer registry linkage	1:2	Municipality (including time for blood collection), age (exact matching)
HIMS (*Wong et al.*, 2014)	Population-based cohort study	Australia	1996-2004	Cancer registry linkage	1:4 [b]	Age at blood collection (±12 months), date of blood collection (± 12 months), fasting status, and diabetes, and requiring that the controls in each matched set be 'alive and at risk' beyond the case's date of diagnosis
HPFS (Platz *et al.*, 2004; Mikkah *et al*., 2007; Shui I *et al*., 2012)	Cohort study of male dentists, optometrists, osteopathic physicians, podiatrists, pharmacists, and veterinarians	USA	1986	Self-report with medical record review; death certificates (for fatal)	1:1	Year of birth (±1 year), date of recruitment (same year), time of blood collection (12 a.m.– 9 a.m., 9 a.m.–12 p.m., 12 p.m.–4 p.m., 4 p.m.–12 a.m.), PSA test before blood draw (y/n), season of blood draw, control participants had ≥1 screening PSA test after the date of blood draw
Janus part 1 (Tuohimaa *et al*., 2004)	Population-based cohort study of participants in county health examinations and blood donors	Norway	1973-onward	Cancer registry linkage	1:4	Age (±2 years), date (± 6 months) and season of blood draw and region
Janus part 2 (Meyer *et al*., 2012)	Participants in population-based health studies and had serum in the Janus Serum Bank	Norway	1981-1991	Cancer registry linkage	1:1	Age at serum sampling (±6 months), date of serum sampling (±2 months) and county of residence (i.e. health examination)

JPHC (Sawada *et al.*, 2017)	Population-based cohort study	Japan	Cohort I – 1990 Cohort II - 1993	Hospital records and cancer registry linkage	1:2	Age (±3 years), area (town or city, and village), public health center area, the date and time of day of blood collection (within 60 days and within 3 hours, respectively) and length of fasting time at blood sampling (within 3 hours).
MCCS (unpublished)[a]	Population-based cohort study	Australia	1991-1994	Cancer registry linkage	1:1 or 1:2 [b]	Age at blood collection (±24 months) and date of blood collection (±24 months), and requiring that the controls in each matched set be 'alive and at risk' beyond the case's date of diagnosis, and that controls had a vitamin D measurement if the matched case had one. Up to 2 controls were matched with each case.
MDCS (Brandstedt *et al*., 2012)	Population-based cohort study	Sweden	1991-1996	Cancer registry linkage	1:1	Calendar time at inclusion (±15 days), age at inclusion (±2 years)
MEC (Park *et al*., 2010)	Population-based cohort study	USA	1993-1996 (Blood collection 2001-2006)	Cancer registry linkage	1:2	Geographical location (California/Hawaii), ethnicity, birth year (±1 year), date blood draw (±6 months), time blood draw (±2 hours), fasting status (0<6, 6-<8, 8-<10, 10+ hours)
PCPT (Schenk *et al*., 2014)	Randomized, placebo-controlled trial of finasteride and prostate cancer	USA	1994-1997	Diagnosed as part of trial protocol. Annual digital rectal examinations and PSA measurements. Biopsy if abnormal DRE or reported PSA level >4.0ng/ml. End-of-study prostate biopsy	1:1	Frequency matched: age (5-year age groups); PCPT treatment arm; positive family history for first-degree relative with prostate cancer. Controls required to have completed end of study biopsy procedure and had no evidence of prostate cancer.
PHS (Gann *et al.*, 1996; Ma *et al.*, 1998; Li *et al.*, 2007)	Randomized trial of aspirin and β-carotene among physicians	USA	1982-onward	Self-report with medical record review	1:1-3	Age (±1 year and ±5 years for older men), smoking status (never, former, current), length of follow-up. Participants included men from both placebo and treatment arms. Cases and controls were not matched by treatment arm but interaction analyses showed no modification of the vitamin D prostate cancer association by treatment arm.
PLCO (Ahn *et al.*, 2008)	Population-based randomized controlled multicenter trial of methods for early detection of cancer of the prostate, lung, colorectal and ovary.	USA	1993-2001	Medical and pathology record review after screening and self-report with medical record review	1:1 frequency matched	Age at cohort entry (5-year intervals), time since initial screening (1-year time window), and calendar year of cohort entry. All study participants selected from trial screening arm, i.e. offered PSA at recruitment and annually for 5 years, plus DRE at recruitment and annually for three years.
ProtecT (Gilbert *et al.*, 2011)	Population-based PSA testing and randomized, controlled trial of treatments of localized prostate cancer	United Kingdom	2001-2009 (Eligible for vitamin D study if recruited 2003-2008)	Diagnosed as part of trial protocol. PSA test at recruitment followed by diagnostic biopsy if PSA ≥3ng/mL	1:1 stratum matched	5-year aged-band (age at PSA test) and GP/family practice, also by time and season of blood draw due to timing of recruitment clinic. (Vitamin D study nested within the prostate cancer detection phase of trial, controls with PSA <3.0ng/mL or raised PSA≥3.0 ng/mL combined with at least 1 negative biopsy)
SELECT (Kristal et al., 2014)	Randomized, placebo-controlled	USA,	July 2011-	Most cases detected by PSA	1:3 for	For each case men were selected for a subcohort at

	trial of selenium and vitamin E in relation to prostate cancer risk	Canada, Puerto Rico	May 2004	and/or DRE screening, which was suggested annually but not required. Pathology reports and slides were obtained where possible.	African American men 1:1.15 for other men	random from the same age/race group. Note that the SELECT intervention assignment was included as a covariate in the original multivariable regression models.
SU.VI.MAX (Deschasaux *et al.*, 2016)	Population-based, double-blind, placebo-controlled, randomized trial of supplementation with antioxidant vitamins and minerals (vitamin C, α-tocopherol, β-carotene, selenium, and zinc)	France	1994	Self-reported in a monthly questionnaire on health-related events or detected through PSA screening of baseline bloods analyzed at the end of trial. PSA values PSA ≥ 4.0 μg/L were followed up.	1:2	Men were matched on age at inclusion (<40/40–44/45–49/50–54/55–65 years), intervention group of the initial SU.VI.MAX trial (placebo/antioxidants) and season of blood draw (a priori defined periods: June–October/November–May)

[a] Unpublished vitamin D and prostate cancer data, Study references: Joshu et al., 2018 for ARIC, Knekt *et al.*, 2008 for FMC and Milne *et al*., 2017 for MCCS

[b] Cases and controls were matched at the pooling center (University of Oxford) from the cohort study data provided.

For expansion of study names see Table 1.

Supplementary Table 2. Assay details for 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D measurements

Study (First author, year)	Sample	25(OH)D assay			1,25(OH)$_2$D assay			Blinded	Same batch[a]
		Method (Manufacturer/ Laboratory)	Intra-assay CV	Inter-assay CV	Method (Manufacturer/ Laboratory)	Intra-assay CV	Inter-assay CV		
ARIC (Unpublished)[d]	Serum	Liquid chromatography-tandem high-sensitivity mass spectrometry (University of Minnesota Molecular Epidemiology and Biomarker Research Laboratory, Minneapolis, MN, USA)	-	20.8%	-	-	-	Yes	Not applicable – cohort
ATBC (Albanes *et al.*, 2011)	Serum	DiaSorin Liaison platform Direct competitive chemiluminescence IA (Heartland Assays, Inc.)	10.5%	12.3%	-	-	-	Yes	Yes
CLUE 1 (Braun *et al.*, 1995)	Serum	RIA (Hollis)	22.01%	11.1%	RRA (Hollis)	21.3%	14.3%	Yes	Yes
EPIC (Travis *et al.*, 2010)	Serum	EIA (Immunodiagnostic Systems, Ltd.)	3.9% - 14.8%	10.8% - 12.0%	-	-	-	Yes	Yes
ESTHER (Ordonez-Mena *et al.*, 2013)		IDS-iSYS (Immunodiagnostic)	<7.3%	<8.9%.	-	-	-	Yes	Not applicable – cohort
FMC (unpublished)[b]	Serum	EIA (Immuno Diagnostic Systems)	-	-	-	-	-	Yes	NK
HIMS (Wong *et al.*, 2014)	Plasma	LIAISON 25 OH Vitamin D TOTAL chemiluminescence IA (DiaSorin Inc.)	-	11.3 – 13.2%	-	-	-	Not stated	Not applicable – cohort
HPFS (Platz *et al.*, 2004; Mikkah *et al.*, 2007; Shui *et al.*, 2012)	EDTA plasma	RIA (Hollis)	5.4% - 14.8%	-	RIA (Hollis)	5.3% -7.3%	-	Yes	Yes
Janus part 1 (Tuohimaa *et al.*, 2004)	Serum	RIA (Incstar)	8.5%	16%	-	-	-	Yes	Yes
Janus part 2 (Meyer *et al.*, 2013)	Serum	HPLC atmospheric pressure chemical ionisation mass spectrometry (Vitas)		7.6% at 47.8 nmol/L, 6.9% at 83 nmol/L	-	-	-	NK	Yes
JPHC (Sawada *et al.*, 2017)	Plasma	RIA (Mitsubishi Kagaku Bio-Clinical Laboratories Inc, Tokyo)	8.9%	-	-	-	-	Yes	Yes
MCCS (unpublished)[b]	Dried blood spots[c]	LC-MS/MS (Queensland Brain Institute, University of Queensland)	-	8.5%	-	-	-	Yes	Not applicable – cohort
MDCS (Brandstedt *et al.*, 2012)	Serum	HPLC (Department of Clinical Chemistry, Skåne University Hospital)	CVs were 8% at 65nmol/L, 6.8% at 190 nmol/L for 25(OH)$_2$D	CVs were 8.5% at 70nmol/L, 7.1% at 210nmol/L for 25(OH)$_3$D	-	-	-	NK	Yes
MEC (Park *et al.*, 2010)	Plasma	IA (Immunodiagnostic Systems, Ltd.)	2%	3%	-	-	-	Not stated	Yes

PCPT (Schenk 2014)	Serum	LIAISON 25 OH Vitamin D TOTAL Assay (DiaSorin Inc.)	CV 8.3%		-	-	-	Yes	'All batched balanced for cases and controls)
PHS (Gann *et al.*, 1996; Ma *et al.*, 1998; Li *et al.*, 2007)	Plasma	RIA (Hollis)	7.9%	-	RIA (Hollis)	8.1%	-	Yes	Yes
PLCO (Ahn *et al.*, 2008)	Serum	RIA (Heartland Assays)	Overall CV 5.9%		-	-	-	Yes	Yes
ProtecT (Gilbert *et al.*, 2011)	Heparin plasma	Tandem MS	-	4.2% - 5.7%	-	-	-	Not stated	Not stated
SELECT (Kristal *et al.*, 2014)	Plasma	LIAISON®25 OH Vitamin D TOTAL Assay (DIaSorin Inc., Stillwater)	-	12.1% for the low QC and 6.9% for the high QC	-	-	-	Yes	Yes
SU.VI.MAX (Deschasaux *et al.*, 2016)	Plasma	Roche Cobas® electrochemiluminescence total 25(OH)D assay (Roche Diagnostics)	4.5%	6.6%	-	-	-	Yes	NK

[a] Cases and controls were assayed in the same batch.

[b] Unpublished vitamin D and prostate cancer data, Study references: Joshu et al., 2018 for ARIC, Knekt *et al.*, 2008 for FMC and Milne *et al*., 2017 for MCCS.

[c] For MCCS, plasma concentrations were estimated from dried blood spots following the approach detailed in Heath AK et al., 2014.

Abbreviations: CV, coefficient of variation; EDTA, Ethylenediaminetetraacetic acid; EIA, enzyme immunoassay; HPLC, high-performance liquid chromatography; IA, immunoassay, type unspecified; LC-MS/MS, liquid chromatography/tandem mass spectrometry; MS, mass spectrometry; NK, not known; RIA, radioimmunoassay; RRA, radioreceptor assay; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D.

For expansion of study names see Table 1.

Supplementary Table 3. Odds ratios for prostate cancer by study-specific tenths of concentration of season-standardized 25(OH)D among cases and their matched controls in prospective studies, conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height and body mass index.

	25(OH)D
Tenth	**OR (95% CI)**
1	1 (reference)
2	1.12 (1.01-1.25)
3	1.13 (1.01-1.25)
4	1.14 (1.02-1.27)
5	1.27 (1.14-1.42)
6	1.16 (1.04-1.29)
7	1.27 (1.14-1.41)
8	1.28 (1.15-1.42)
9	1.24 (1.12-1.39)
10	1.34 (1.20-1.49)
***P* for trend**	<0.001

Supplementary Table 4. Multivariable-adjusted odds ratios (95% confidence intervals) for prostate cancer in prospective studies by pre-specified categories of season-standardized 25-hydroxyvitamin D concentration

Category (nmol/L)	Cases/Controls	Season-standardized 25-hydroxyvitamin D	
		OR	(95% CI)
<30	919/1431	0.84	(0.76-0.93)
30-49	3043/5163	0.89	(0.84-0.95)
50-74 (reference)	5318/8018	1.00	(ref)
≥ 75	4182/5649	1.07	(1.00-1.13)

The odds ratios were conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height, and body mass index. Median concentrations of season-standardized 25(OH)D in each group were 23.8, 42.0, 61.5 and 89.7 nmol/L, respectively.

Supplementary Table 5A. Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and season-standardized 1,25(OH)$_2$D, among cases and their matched controls in prospective studies. Data on both analytes were available for a total of 1885 cases and 2114 controls from 3 studies (CLUE I, HPFS, PHS).

		Odds ratio (95% confidence interval)		
		Third of 25(OH)D		
		1	2	3
Third of 1,25(OH)$_2$D	**1**	1 (reference)	0.96 (0.72-1.27)	1.00 (0.75-1.34)
	2	0.91 (0.69-1.20)	1.32 (1.01-1.73)	1.16 (0.87-1.54)
	3	1.12 (0.83-1.52)	1.21 (0.91-1.59)	1.06 (0.81-1.39)
***P* for interaction**	**0.23**			

Supplementary Table 5B. Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and IGF-I among cases and their matched controls in prospective studies. Data on both analytes were available for a total of 3050 cases and 4354 controls from 7 studies (EPIC phase 1, HIMS, HPFS, MEC, PCPT, PHS, SU.VI.MAX).

		Odds ratio (95% confidence interval)		
		Third of 25(OH)D		
		1	2	3
Third of IGF-I	**1**	1 (reference)	1.12 (0.91-1.39)	1.26 (1.02-1.56)
	2	1.07 (0.87-1.33)	1.19 (0.96-1.46)	1.22 (0.99-1.50)
	3	1.10 (0.88-1.37)	1.27 (1.03-1.57)	1.31 (1.06-1.61)
***P* for interaction**	**0.95**			

Supplementary Table 5C. Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and IGFBP-3 among cases and their matched controls in prospective studies. Data on both analytes were available for a total of 2971 cases and 4212 controls from 6 studies (EPIC phase 1, HIMS, HPFS, MEC, PCPT, PHS).

		Odds ratio (95% confidence interval)		
		Third of 25(OH)D		
		1	2	3
Third of IGFBP-3	**1**	1 (reference)	1.05 (0.84-1.30)	1.13 (0.91-1.42)
	2	1.09 (0.87-1.35)	1.32 (1.06-1.63)	1.38 (1.11-1.70)
	3	1.18 (0.95-1.48)	1.31 (1.06-1.63)	1.38 (1.11-1.72)
***P* for interaction**	**0.91**			

Supplementary Table 5D. Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and testosterone among cases and their matched controls in prospective studies. Data on both analytes were available for a total of 3003 cases and 6062 controls from 8 studies (EPIC phase 1, FMC, HIMS, JPHC, Janus part 1, MEC, PCPT, PHS).

		Odds ratio (95% confidence interval)		
		Third of 25(OH)D		
		1	2	3
Third of testosterone	**1**	1 (reference)	1.03 (0.85-1.24)	1.28 (1.05-1.55)
	2	0.98 (0.80-1.19)	1.05 (0.86-1.28)	1.27 (1.05-1.54)
	3	0.98 (0.80-1.20)	1.06 (0.87-1.29)	1.08 (0.89-1.30)
***P* for interaction**	**0.55**			

Supplementary Table 5E. Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and free testosterone among cases and their matched controls in prospective studies. Data on both analytes were available for a total of 2969 cases and 6062 controls from 8 studies (EPIC phase 1, FMC, HIMS, JPHC, Janus part 1, MEC, PCPT, PHS).

		Odds ratio (95% confidence interval)		
		Third of 25(OH)D		
		1	2	3
Third of free testosterone	**1**	1 (reference)	1.07 (0.88-1.30)	1.29 (1.06-1.57)
	2	1.08 (0.88-1.31)	1.10 (0.91-1.34)	1.27 (1.05-1.55)
	3	0.98 (0.80-1.21)	1.07 (0.87-1.31)	1.18 (0.97-1.43)
***P* for interaction**	**0.95**			

Supplementary Table 5F. Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and SHBG among cases and their matched controls in prospective studies. Data on both analytes were available for a total of 3088 cases and 6254 controls from 8 studies (EPIC phase 1, FMC, HIMS, JPHC, Janus part 1, MEC, PCPT, PHS).

		Odds ratio (95% confidence interval)		
		Third of 25(OH)D		
		1	2	3
Third of SHBG	**1**	1 (reference)	0.97 (0.80-1.17)	1.14 (0.94-1.38)
	2	0.91 (0.75-1.10)	0.96 (0.80-1.16)	1.15 (0.95-1.38)
	3	0.81 (0.66-0.99)	0.93 (0.76-1.13)	1.03 (0.85-1.25)
***P* for interaction**	**0.78**			

Supplementary Table 5G. Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and PSA among cases and their matched controls in prospective studies. Data on both analytes were available for a total of 4470 cases and 5111 controls from 8 studies (EPIC phase 1, Janus part 1, MEC, PCPT, PHS, PLCO, SELECT, SU.VI.MAX)

		Odds ratio (95% confidence interval)		
		Third of 25(OH)D		
		1	2	3
Third of PSA	**1**	1 (reference)	1.38 (1.00-1.88)	1.09 (0.78-1.52)
	2	3.01 (2.26-4.01)	3.13 (2.36-4.16)	3.33 (2.51-4.43)
	3	14.1 (10.8-18.4)	16.0 (12.2-20.9)	17.6 (13.5-23.1)
***P* for interaction**	**0.36**			

Supplementary Table 6. Partial correlations among controls in all studies between log-transformed concentrations of circulating 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and other analytes, standardized within each study and adjusted for age at blood collection (5 age-groups).

	Observed 25(OH)D [c]	Season-standardized 25(OH)D	$1,25(OH)_2D$	Season-standardized $1,25(OH)_2D$
Season-standardized 25(OH)D	0.95[b]	-	-	-
	(21,701)			
$1,25(OH)_2D$	0.14[b]	0.13[b]	-	-
	(3398)			
Season-standardized $1,25(OH)_2D$	0.12[b]	0.13[b]	0.99[b]	-
	(3398)		(3384)	
IGF-I	0.05[b]	0.06[b]	0.03	0.04
	(5660)		(1893)	
IGF-II	0.06[a]	0.07[b]	0.03	0.03
	(3061)		(1101)	
IGFBP-1	0.11[b]	0.12[b]	0.10[a]	0.11[a]
	(2761)		(834)	
IGFBP-2	0.10[b]	0.10[b]	-0.04	-0.04
	(1996)		(1019)	
IGFBP-3	0.05[b]	0.06[b]	0.06[a]	0.07[a]
	(5326)		(1882)	
SHBG	0.08[b]	0.08[b]	0.04	0.04
	(6307)		(219)	
Testosterone	0.09[b]	0.10[b]	-0.01	-0.01
	(6256)		(219)	
Free testosterone	0.05[b]	0.06[b]	-0.02	-0.01
	(6235)		(219)	
Estradiol	-0.01	-0.01	(<10 obs)	(<10 obs)
	(2224)			
Free Estradiol	-0.04[a]	-0.04[a]	(<10 obs)	(<10 obs)
	(2220)			
Insulin	-0.08[b]	-0.08[b]	-0.09	-0.11
	(3441)		(33)	
C-peptide	-0.11[b]	-0.12[b]	-0.13[b]	-0.13[b]
	(2166)		(849)	
Lycopene	0.07[b]	0.05[a]	-0.00	0.00
	(3483)		(1192)	
Prostate-specific antigen	0.01	0.01	0.01	0.01
	(6768)		(1816)	

[a] Two -sided significance level P <0.05

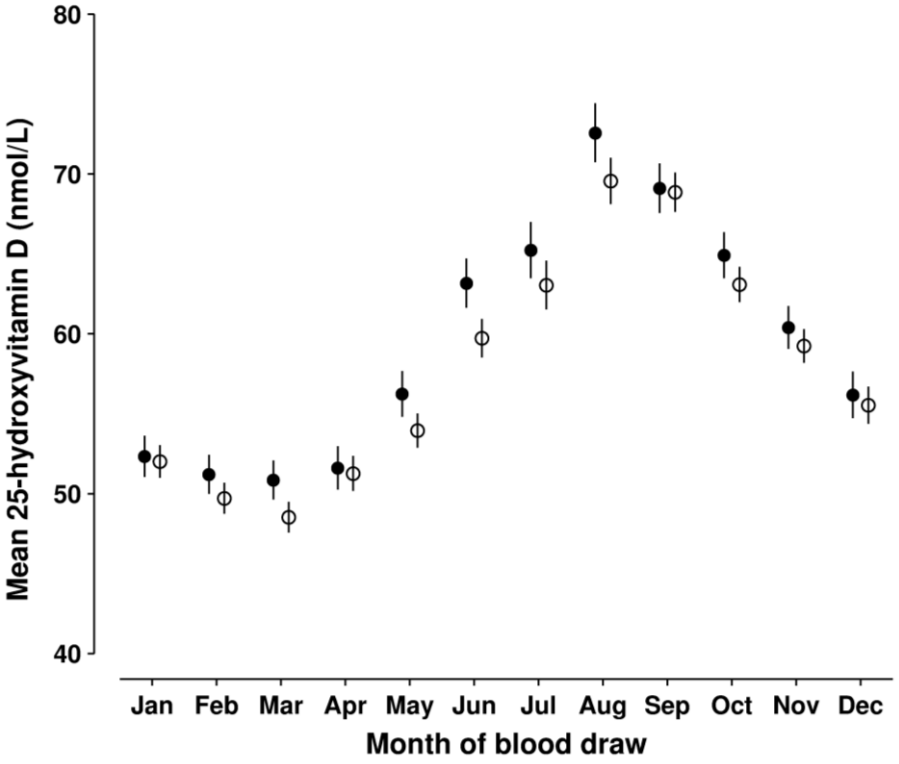
[b] Two-sided significance level P <0.001

[c] Numbers in parentheses are numbers of controls with data on both analytes

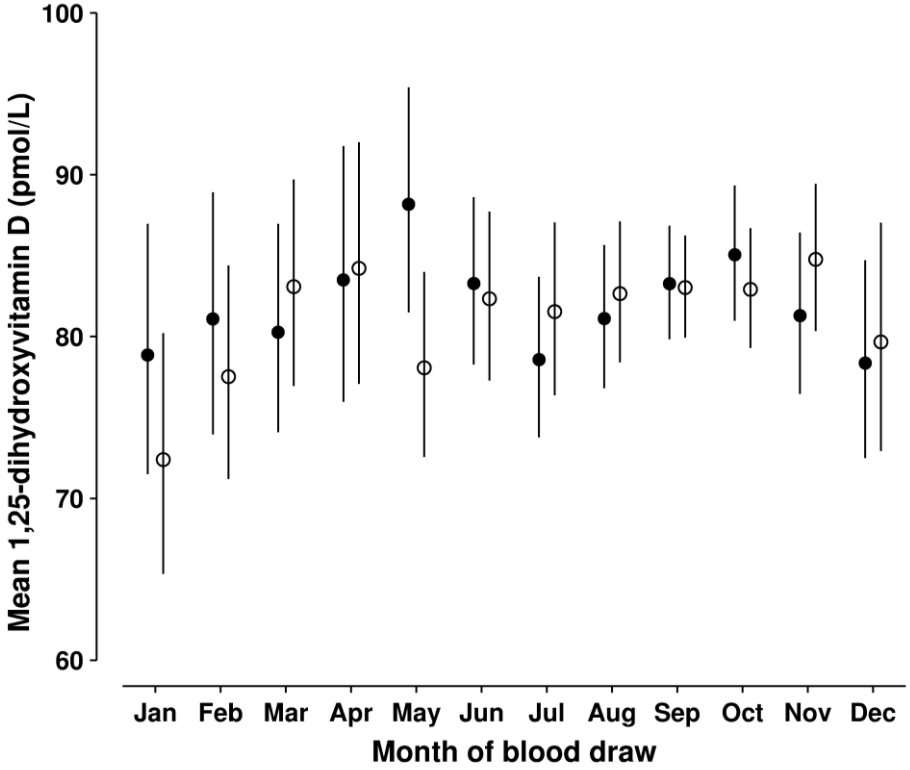
Abbreviations: IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; SHBG, sex hormone binding globulin;

25(OH)D,25-hydroxyvitamin D ; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D.

A. 25-hydroxyvitamin D (nmol/L)

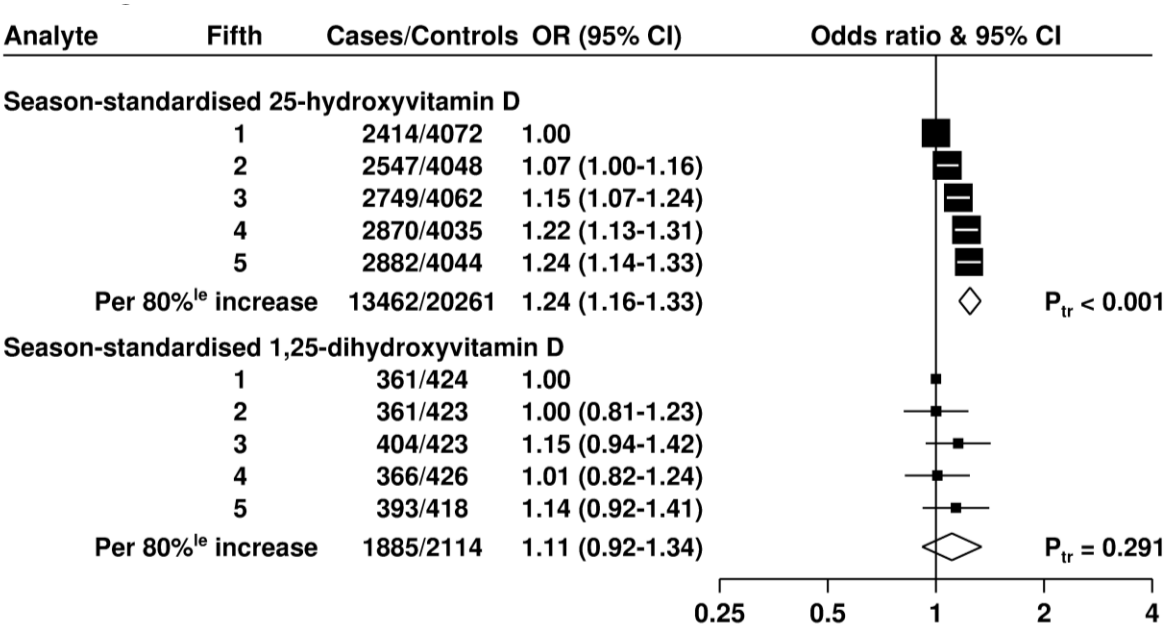


B. 1,25 dihydroxyvitamin D (pmol/L)

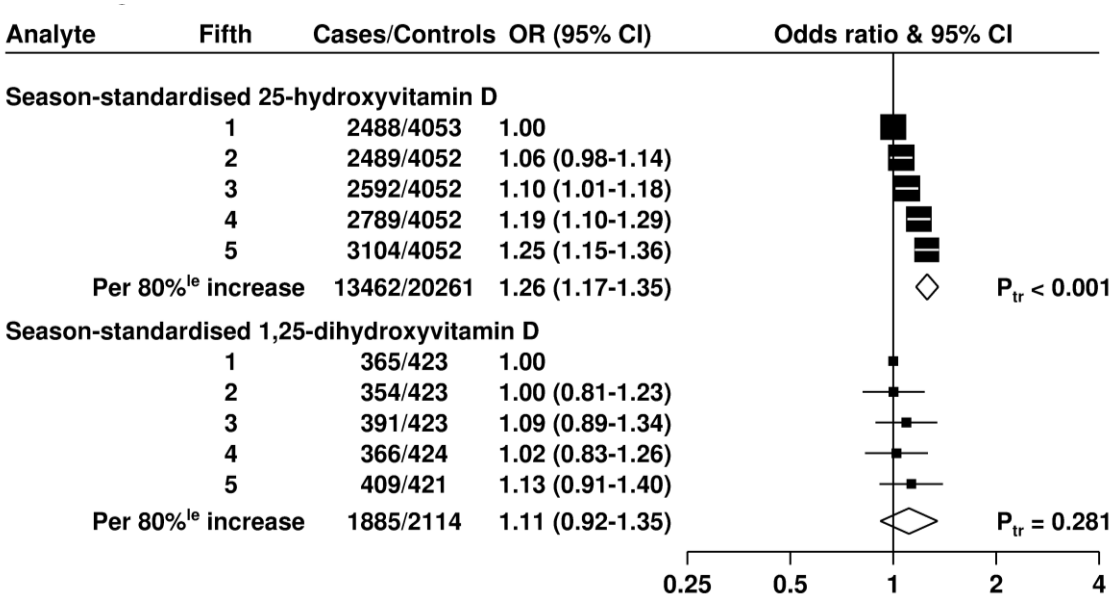


Supplementary Figure 1. Geometric mean concentrations (95% confidence intervals) of 25-hydroxyvitamin D (nmol/L) and 1,25 dihydroxyvitamin D (pmol/L) for all prospective studies by month of blood collection (corrected for hemisphere so that January is treated as July, February as August, and so on for the HIMS and MCCS studies) and case-control status, adjusted for study and age at blood collection. The geometric means among case patients are depicted by solid circles and among control participants by open circles.

A. Study-specific cut-points

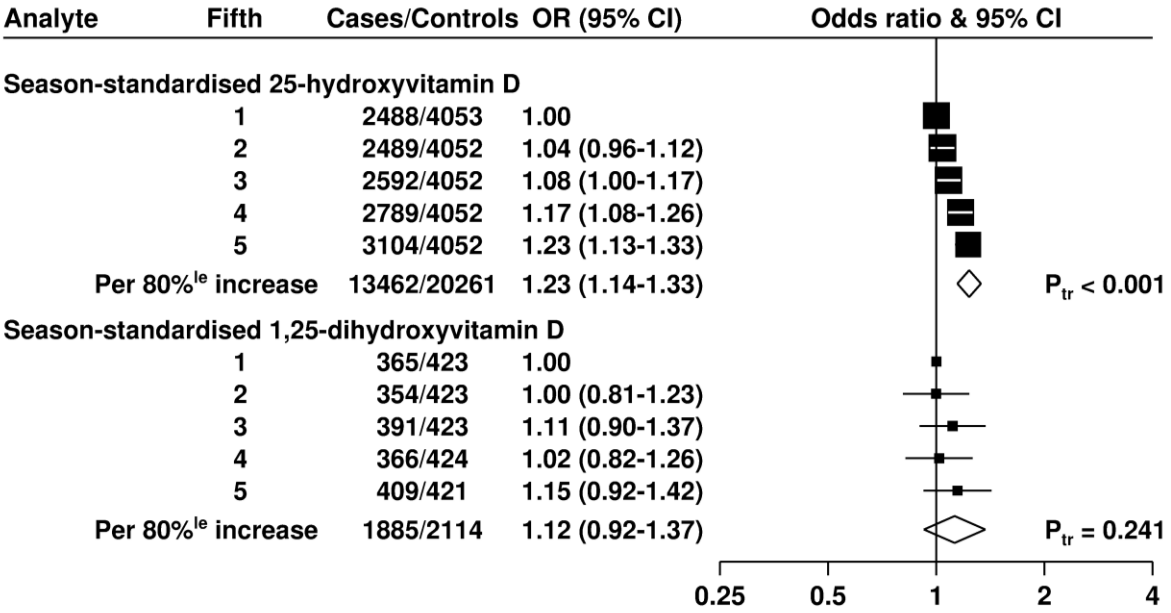


B. Overall cut-points (across all prospective studies combined)



Supplementary Figure 2. Odds ratios (95% confidence intervals) for prostate cancer associated with study-specific and overall fifths of concentrations of season-standardised 25-hydroxyvitamin D and 1,25-dihydroxyvitamind D concentration in prospective studies.

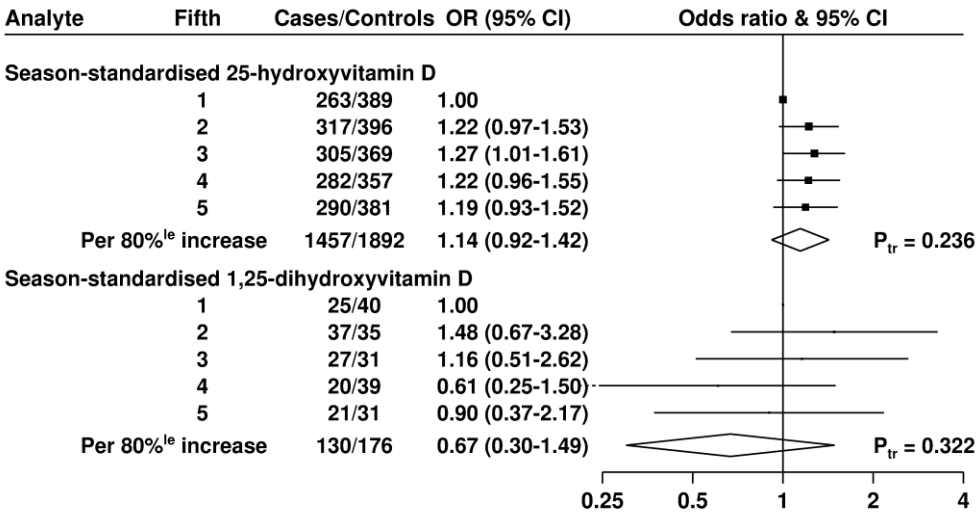
Estimates are from logistic regression conditioned on the matching variables within each study, and without mutual adjustment for the other analyte. *P*trend was calcuated by replacing the fifths of concentration with a continuous variable that was scored 0, 0.25, 0.5, 0.75 and 1 in the conditional logistic regression model. Median concentrations in each fifth (using overall cut-points) are: 33.6, 48.6, 60.1, 73.3 and 96.1 nmol/L, respectively, for season-standardized 25-hydroxyvitamin D and 57.2, 72.8, 84.7, 97.7 and 122.2 pmol/L, respectively, for season-standardized 1,25-dihydroxyvitamind D. Abbreviations: 80%le = 80 percentile; CI = confidence interval; P_{tr} = *P*trend.



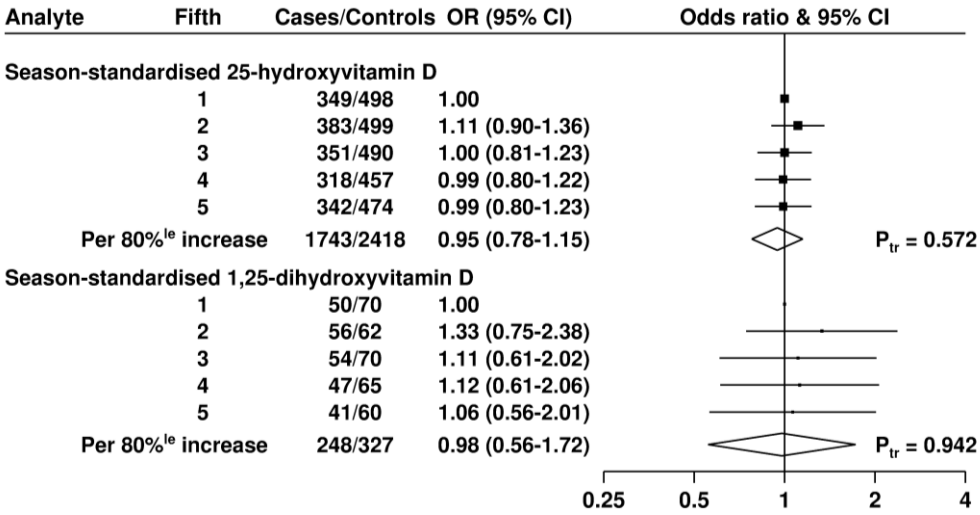
Supplementary Figure 3. Odds ratios (95% confidence intervals) for prostate cancer associated with overall (across all prospective studies combined) fifths of season-standardized 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentration in prospective studies.

Estimates are from logistic regression conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height and body mass index. *P*trend was calculated by replacing the fifths of vitamin D with a continuous variable that was scored as 0, 0.25, 0.5, 0.75 and 1 in the conditional logistic regression model. Median concentrations in each fifth are: 33.2, 48.6, 60.3, 74.0 and 97.8 nmol/L, respectively, for season-standardized 25-hydroxyvitamin D and 57.2, 72.8, 84.7, 97.7 and 122.2 pmol/L, respectively, for season-standardized 1,25-dihydroxyvitamind D. Abbreviations: 80%le= 80 percentile; CI = confidence interval; Ptr = *P*trend.

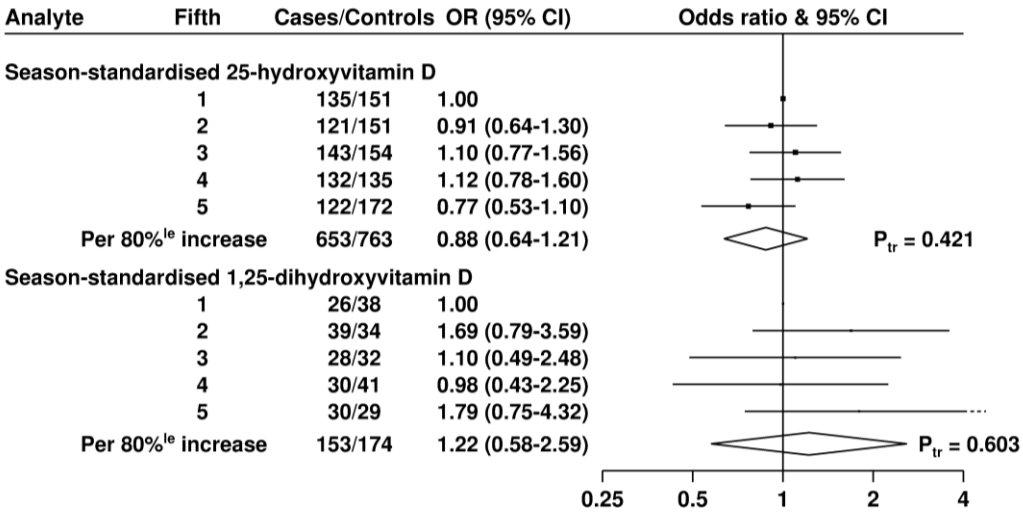
A. Advanced stage prostate cancer [a]



B. Aggressive prostate cancer [b]



C. High grade prostate cancer [c]



Supplementary Figure 4. Odds ratios (95% confidence intervals) for advanced stage, aggressive and high grade prostate cancer associated with study-specific fifths of season-standardized 25-hydroxyvitamin in prospective studies.

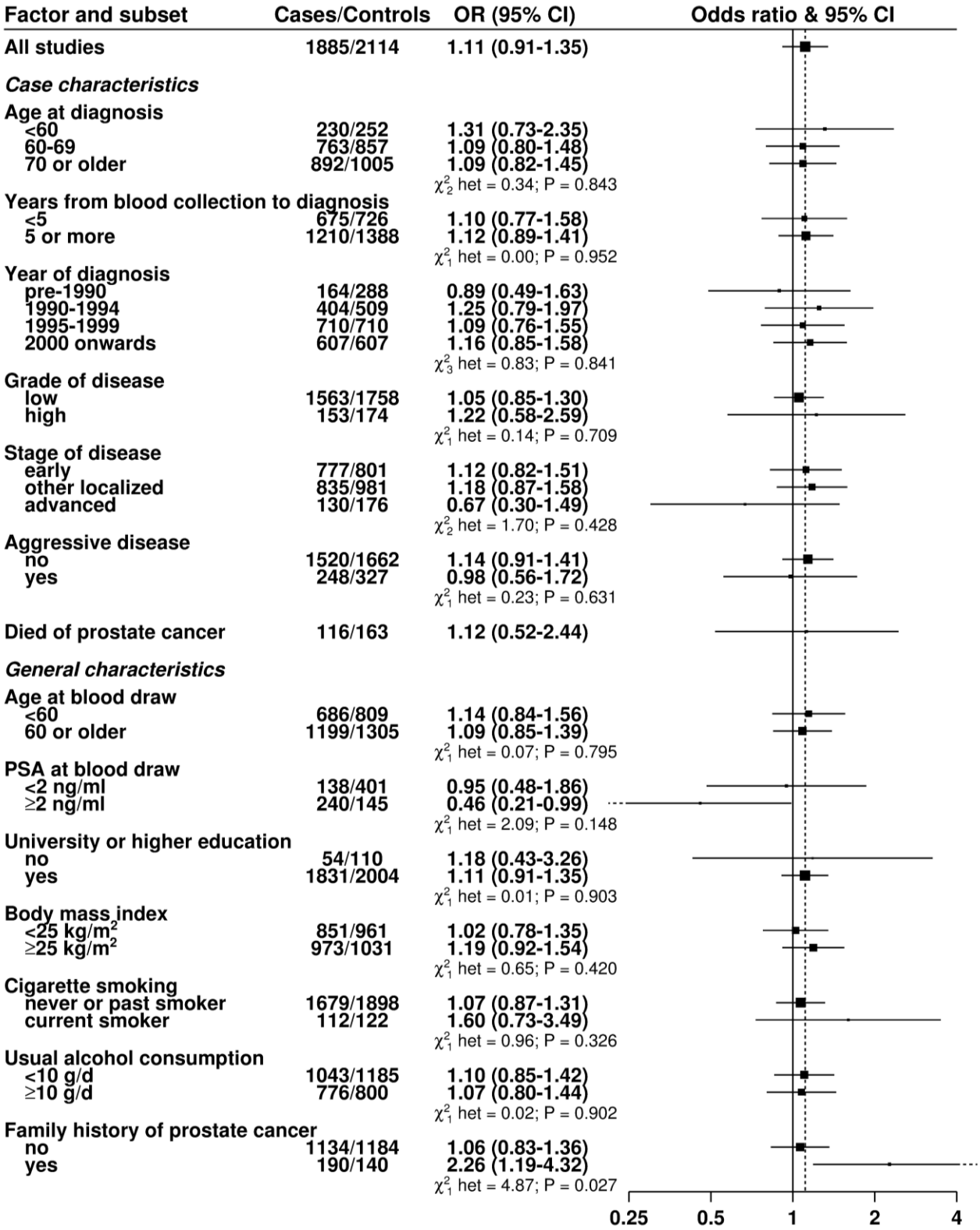
Estimates are from logistic regression conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height and body mass index[a]. Abbreviations: 80%le= 80 percentile; CI = confidence interval; Ptr = *P*trend.

*P*trend (P_{tr}) was calculated by replacing the fifths of vitamin D with a continuous variable that was scored as 0, 0.25, 0.5, 0.75 and 1 in the conditional logistic regression model.

[a] Prostate cancer was defined as being "advanced" stage if it was tumor-node-metastasis (TNM) stage T3 or T4 and/or N1+ and/or M1, stage III–IV, or the equivalent (that is, a tumor extending beyond the prostate capsule and/or lymph node involvement and/or distant metastases).

[b] Prostate cancer was defined as being aggressive disease if it was TNM stage T4 and/or N1+ and/or M1 and/or stage IV disease and/or death from prostate cancer.

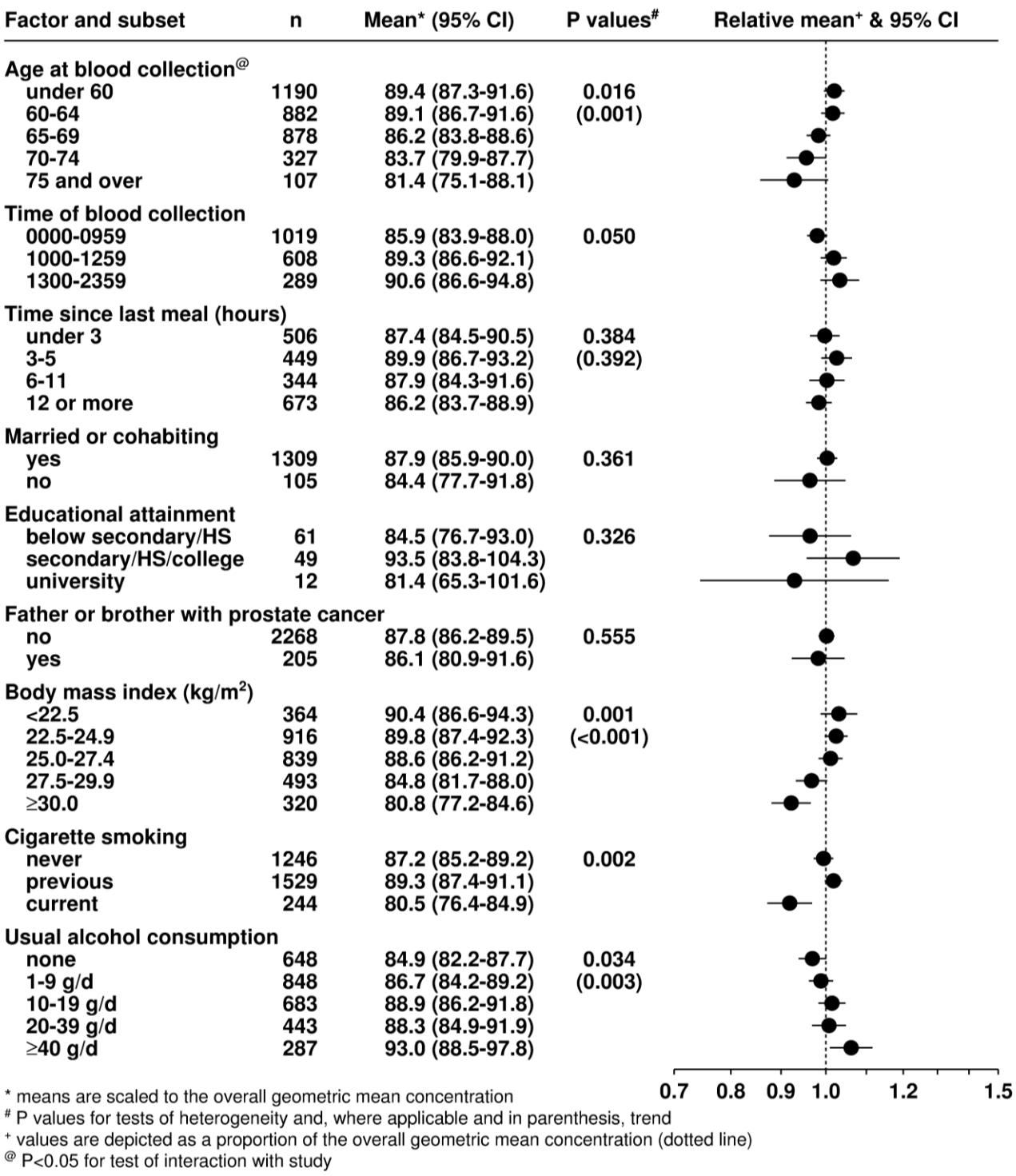
[c] Prostate cancer was defined as high-grade if the Gleason sum was at least 8 or equivalent (i.e. undifferentiated).



Supplementary Figure 5. Odds ratios (95% confidence intervals) for prostate cancer associated with an 80 percentile increase in season-standardized 1,25 dihydroxyvitamin D in prospective studies for selected subgroups.

The odds ratios were conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height and body mass index. Tests for heterogeneity for the case-defined factors were obtained by fitting separate models for each subgroup and assuming independence of the ORs using a method analogous to a meta-analysis. Tests for heterogeneity for the other factors were assessed with a χ2-test of interaction between the subgroup and continuous trend test variable. Note that the number of cases for each tumor subtype may be fewer than shown in the baseline tables since here the analysis for each subgroup of a case-defined factor is restricted to complete matched sets for each category of the factor in turn; some matched sets contain a mixture of subtypes and while controls are allocated case-defined characteristics in equal proportion to the cases, $1,25(OH)_2D$ may be unknown for some participants, leading to incomplete matched sets.

Stage (early, T1 and/or stage I; other localized, T2/N0/M0 and/or stage II, and advanced, T3-T4/N1/M1 and/or stage III-IV), grade (low-intermediate, Gleason sum was < 8 or equivalent; high, Gleason sum was ≥ 8 or equivalent, and aggressive (T4/N1/M1 and/or stage IV and/or prostate cancer death).



Supplementary Figure 6. Geometric mean concentrations (95% confidence intervals) of season-standardized 1,25 dihydroxyvitamin D (pmol/L) for controls from all studies by various factors, adjusted for study and age at blood collection.

Means are scaled to, and depicted as a proportion of, the overall geometric mean concentration (dotted line). P values are for tests of heterogeneity and, where applicable in parentheses, trend.