**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 Published and unpublished studies were eligible for the current collaborative individual participant meta-analysis if they had data on pre-diagnostic circulating concentrations of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cohort studies by the date of dataset closure (May 2018):the Atherosclerosis Risk in Communities (ARIC) Study (unpublished, study described in [8], Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) [9]; Campaign Against Cancer and Stroke (“Give Us a Clue to Cancer”)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Prospective Investigation into Cancer and Nutrition (EPIC) [12]; Finnish Mobile Clinic Health Examination Survey (FMC) (unpublished, study described in [13]); Health Professionals Follow-up Study (HPFS) [14-16]; Health In Men Study (HIMS) [17]; the Janus study that formed part of the of the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2) [19]; Japan Public Health Center–based Prospective (JPHC) Study [20];Melbourne Collaborative Cohort Study (MCCS) (unpublished, study described in [21]);Malmö Diet and Cancer Study (MDCS) [22]; Multiethnic Cohort (MEC) [23]; Prostate Cancer Prevention Trial (PCPT) [24]; Physicians’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)2D 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)2D 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)2D, 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 10th and 90th 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 χ2 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 χ2-test of interaction between subgroup and the continuous trend test variable.

In order to assess potential effect modification with different biomarkers, a χ2-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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**Supplementary tables and figures**

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| **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 trial of selenium and vitamin E in relation to prostate cancer risk | USA, Canada, Puerto Rico | July 2011- May 2004 | Most cases detected by PSA and/or DRE screening, which was suggested annually but not required. Pathology reports and slides were obtained where possible. | 1:3 for African American men  1:1.15 for other men | For each case men were selected for a subcohort at 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. | | | | | | |
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| **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)2D assay** | | | **Blinded** | **Same batcha** |
|  | **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 spotsc | 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)2D | | CVs were 8.5% at 70nmol/L, 7.1% at 210nmol/L for 25(OH)3D |  | - | - | - | 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. | | | | | | | | | | | |

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| **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 |

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| **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. | | | |

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| **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)2D, 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)2D** | **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** |  |  |  |

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| **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** |  |  |  |

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| **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** |  |  |  |

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| **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** |  |  |  |

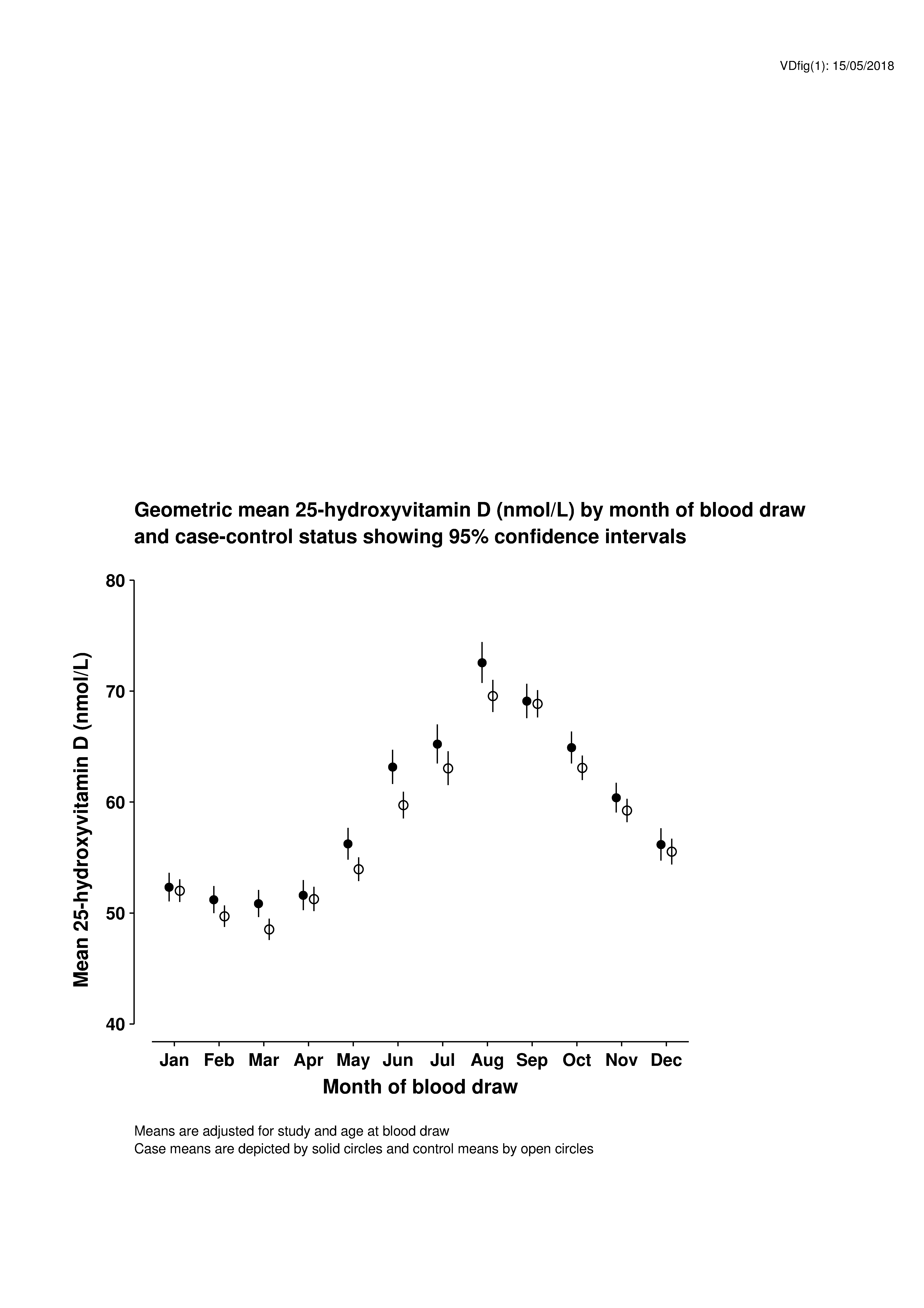
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| **Supplementary Table 5E.** Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and free testosterone among cases and their matched controls in prospective studies. Data 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** |  |  |  |

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| **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** |  |  |  |

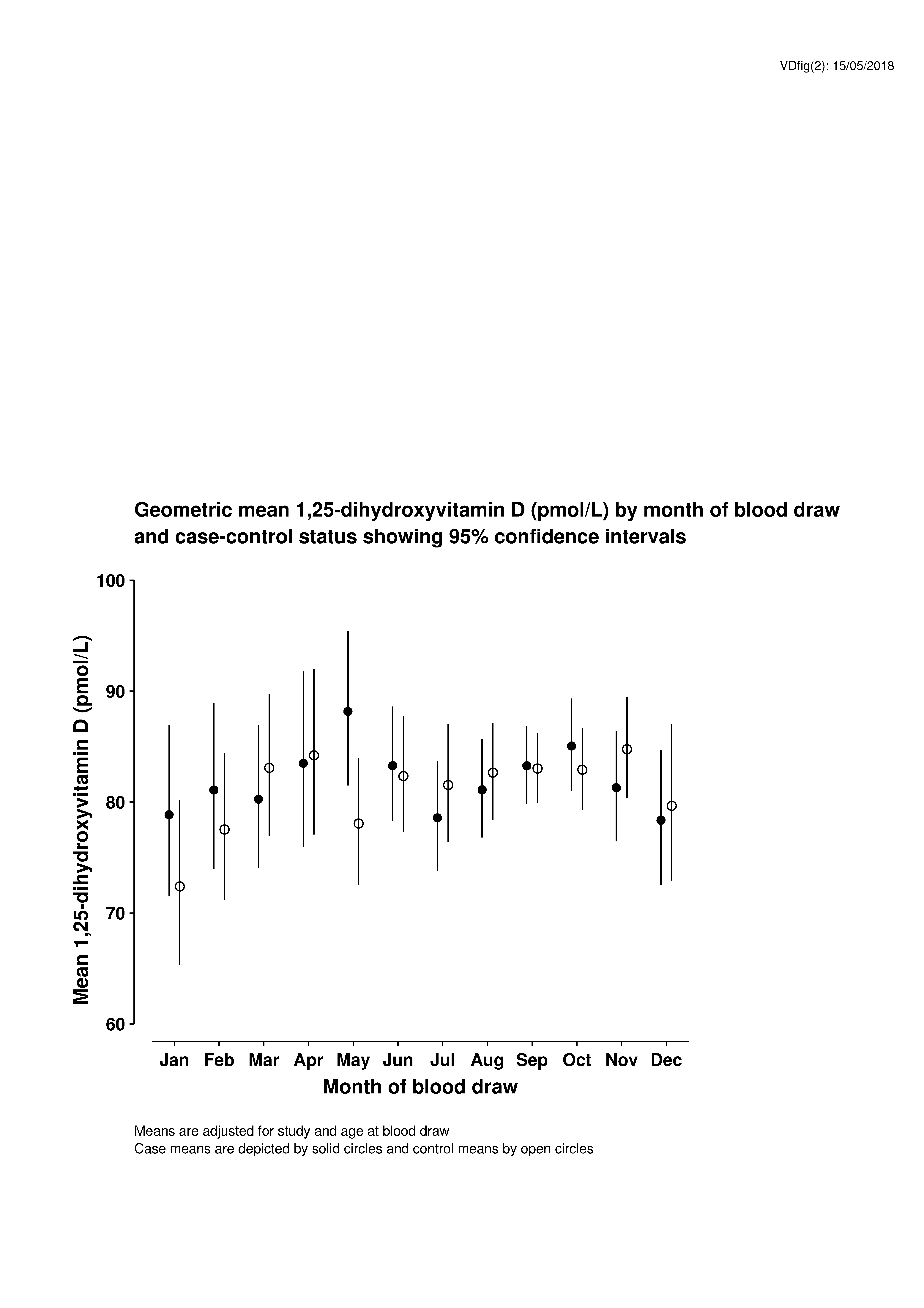
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| **Supplementary Table 5G.** Multivariable-adjusted odds ratios for prostate cancer by study-specific thirds of concentration of season-standardized 25(OH)D and PSA among cases and their matched controls in prospective studies. Data on both analytes 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** |  |  |  |

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

1. **25-hydroxyvitamin D (nmol/L)**

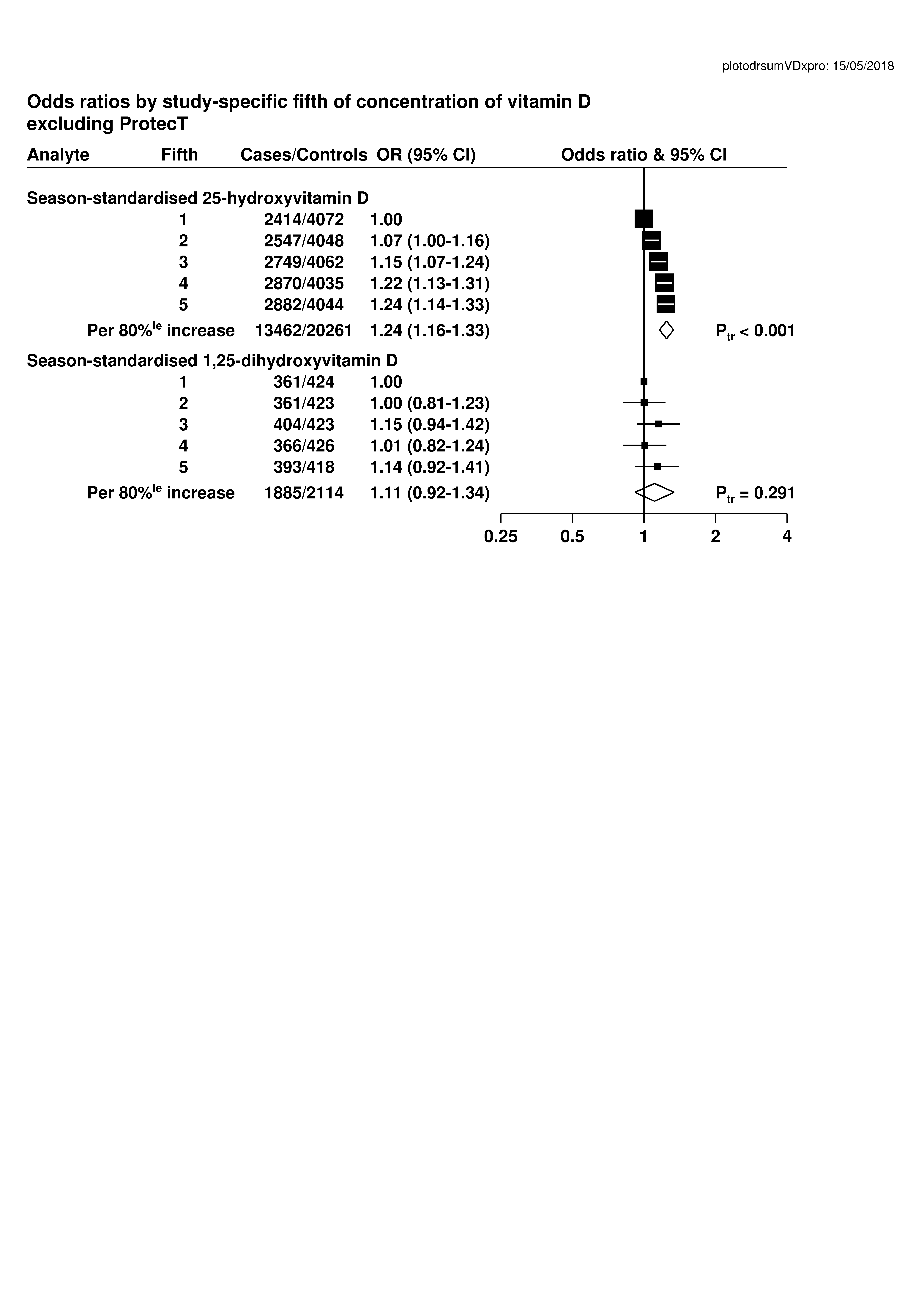


1. **1,25 dihydroxyvitamin D (pmol/L)**

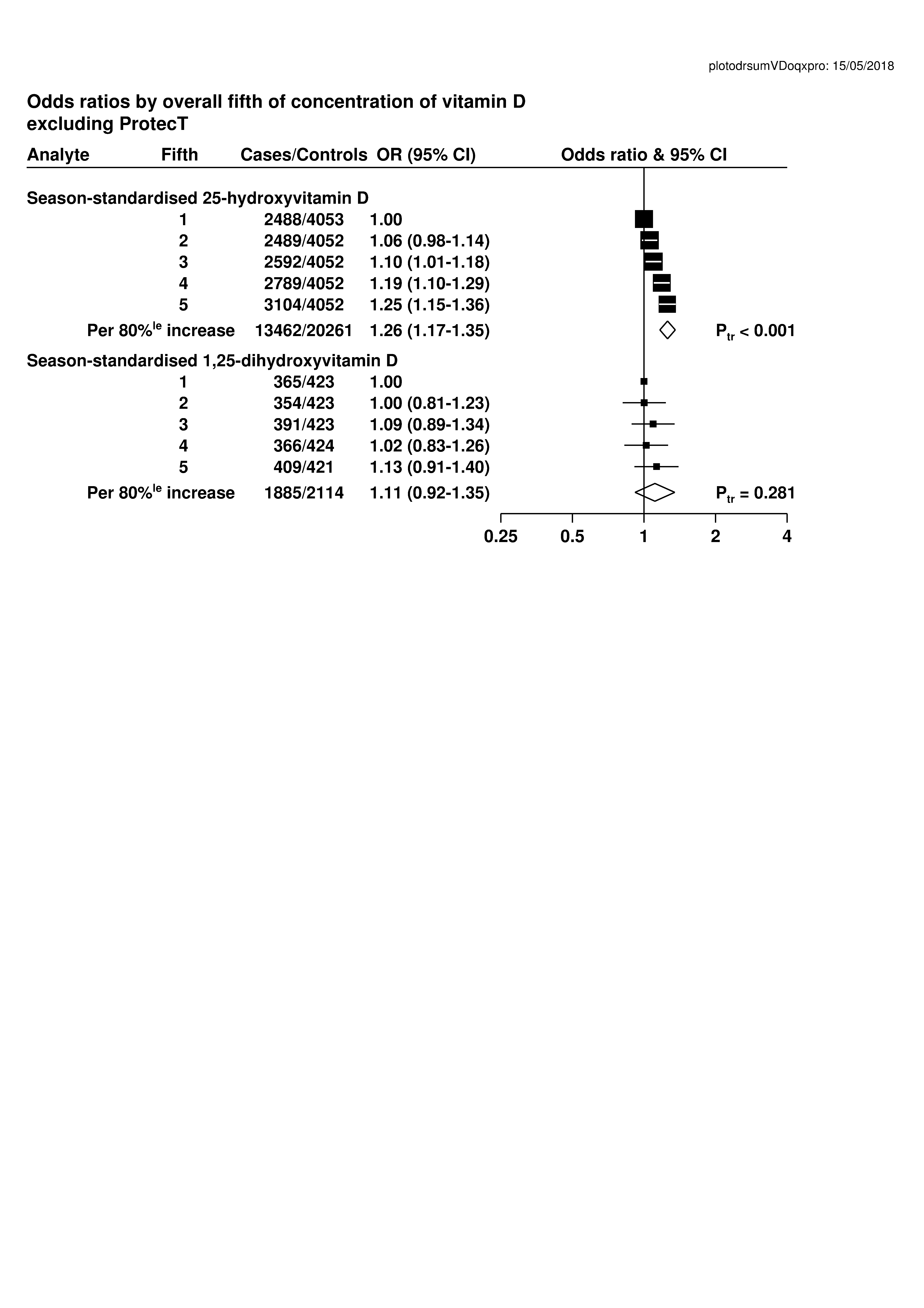


**Supplementary Figure 1.** Geometric mean concentrations (95% confidence intervals) of 25-hydroxyvitamin D (nmol/L) and 1,25 dihydroxyvitamin D (pmol/L) for all prospective studies by month of blood collection (corrected for hemisphere so that January is treated as July, February as August, and so on 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.

1. **Study-specific cut-points**

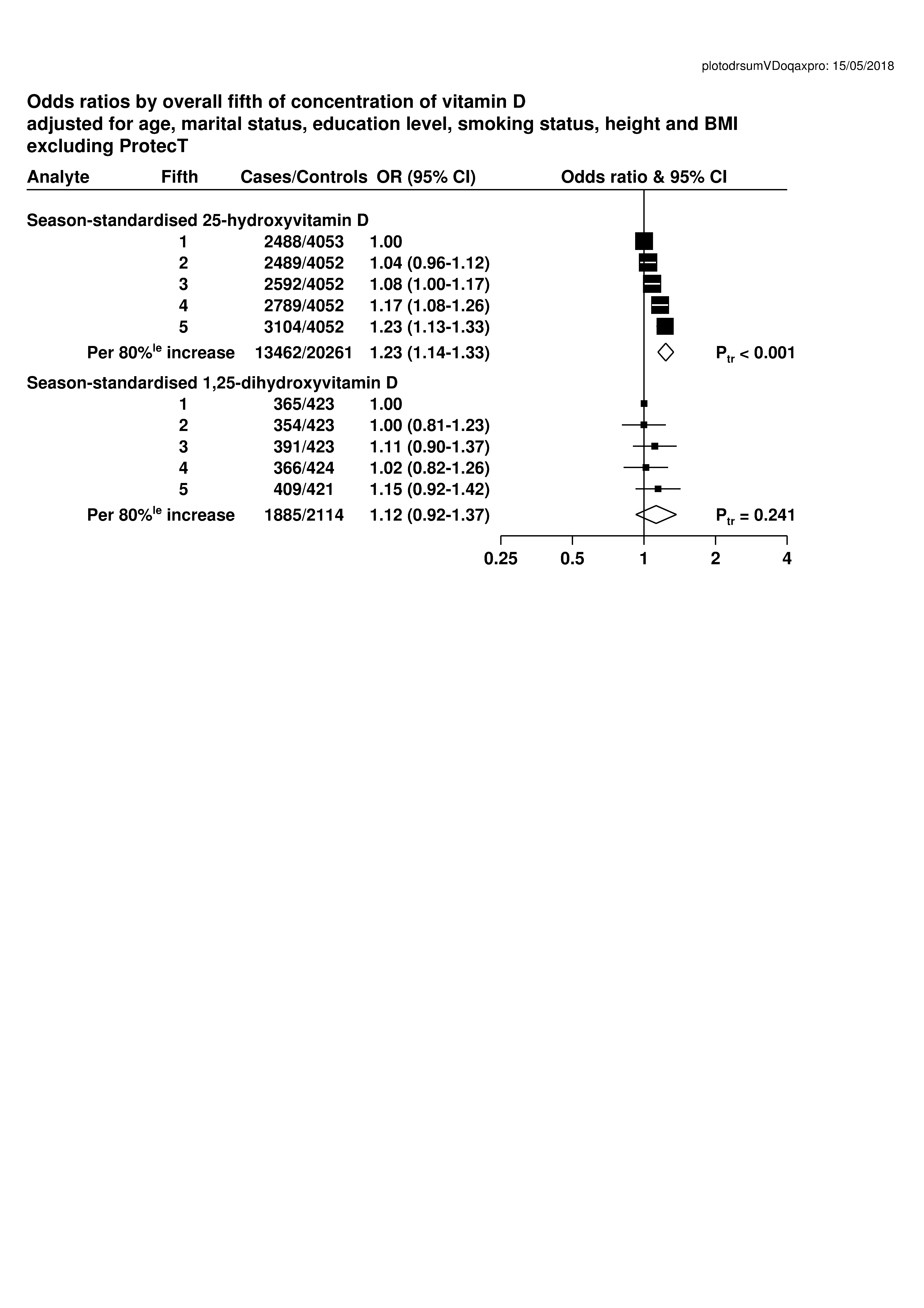
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1. **Overall cut-points (across all prospective studies combined)**

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**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.

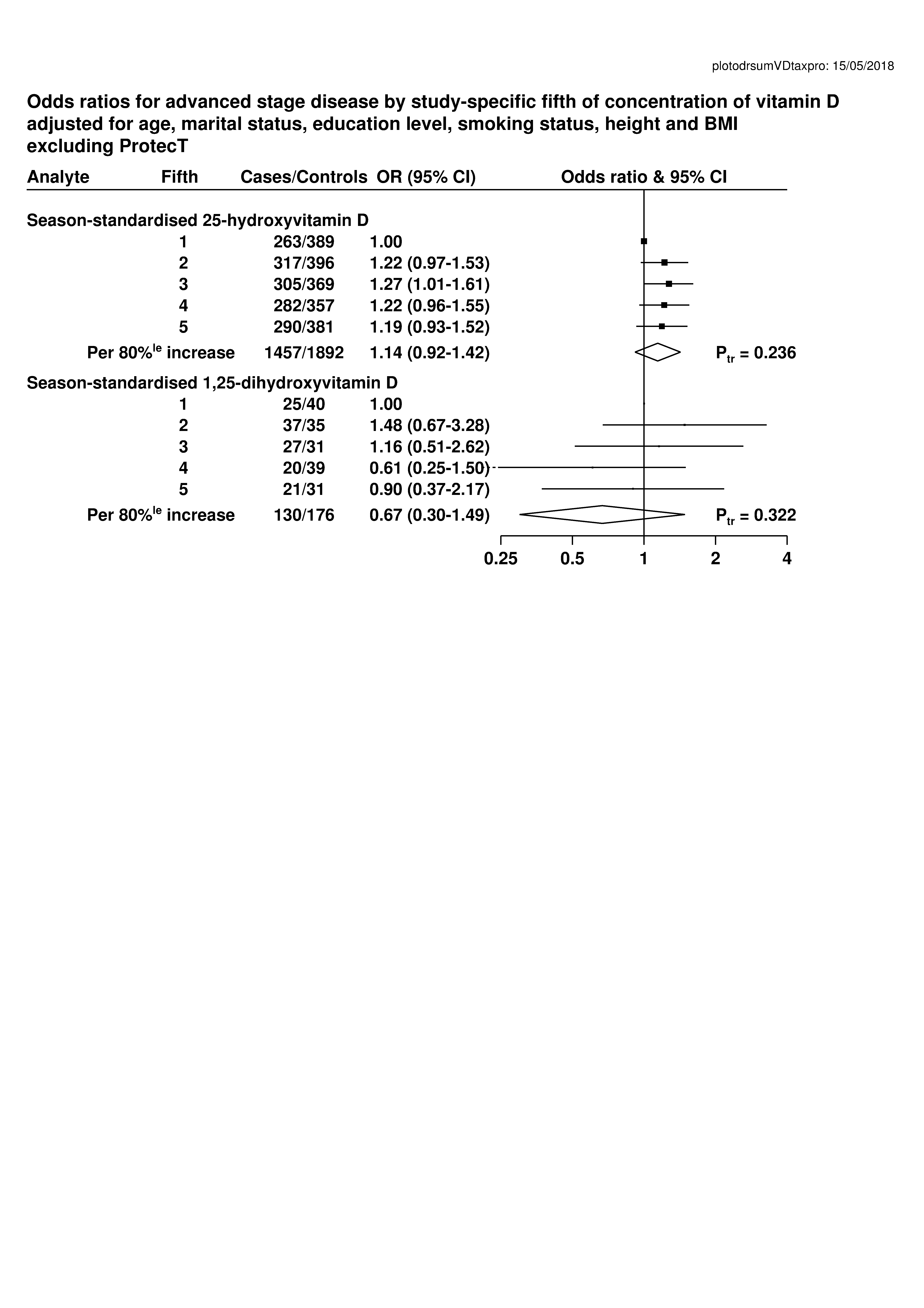
Estimates are from logistic regression conditioned on the matching variables within each study, and without mutual adjustment for the other analyte. *P*trend was calcuated by replacing the fifths of concentration with a continuous variable that was scored 0, 0.25, 0.5, 0.75 and 1 in the conditional logistic 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; Ptr = *P*trend.



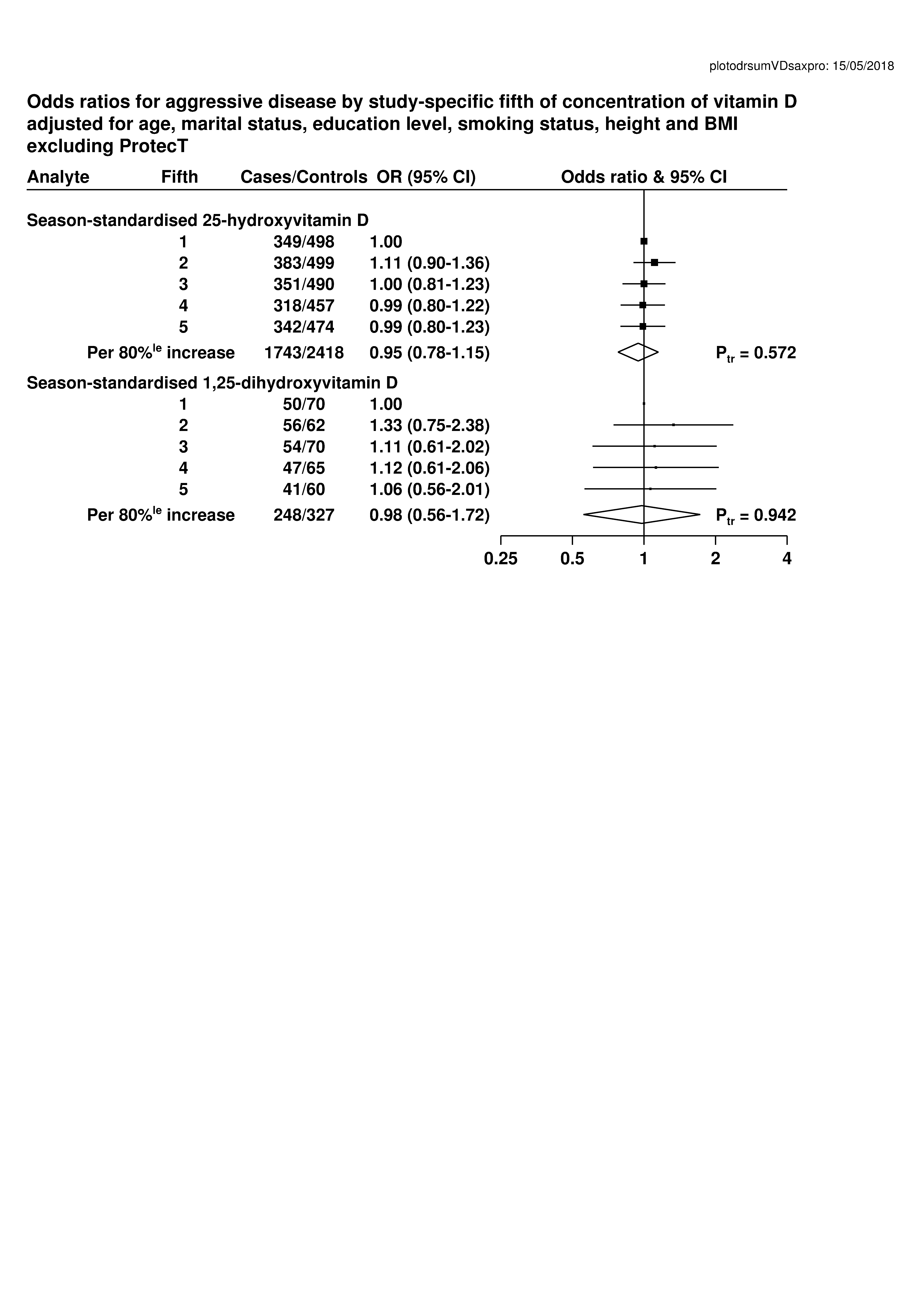
**Supplementary Figure 3.** Odds ratios (95% confidence intervals) for prostate cancer associated with overall (across all prospective studies combined) fifths of season-standardized 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentration in prospective studies.

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.

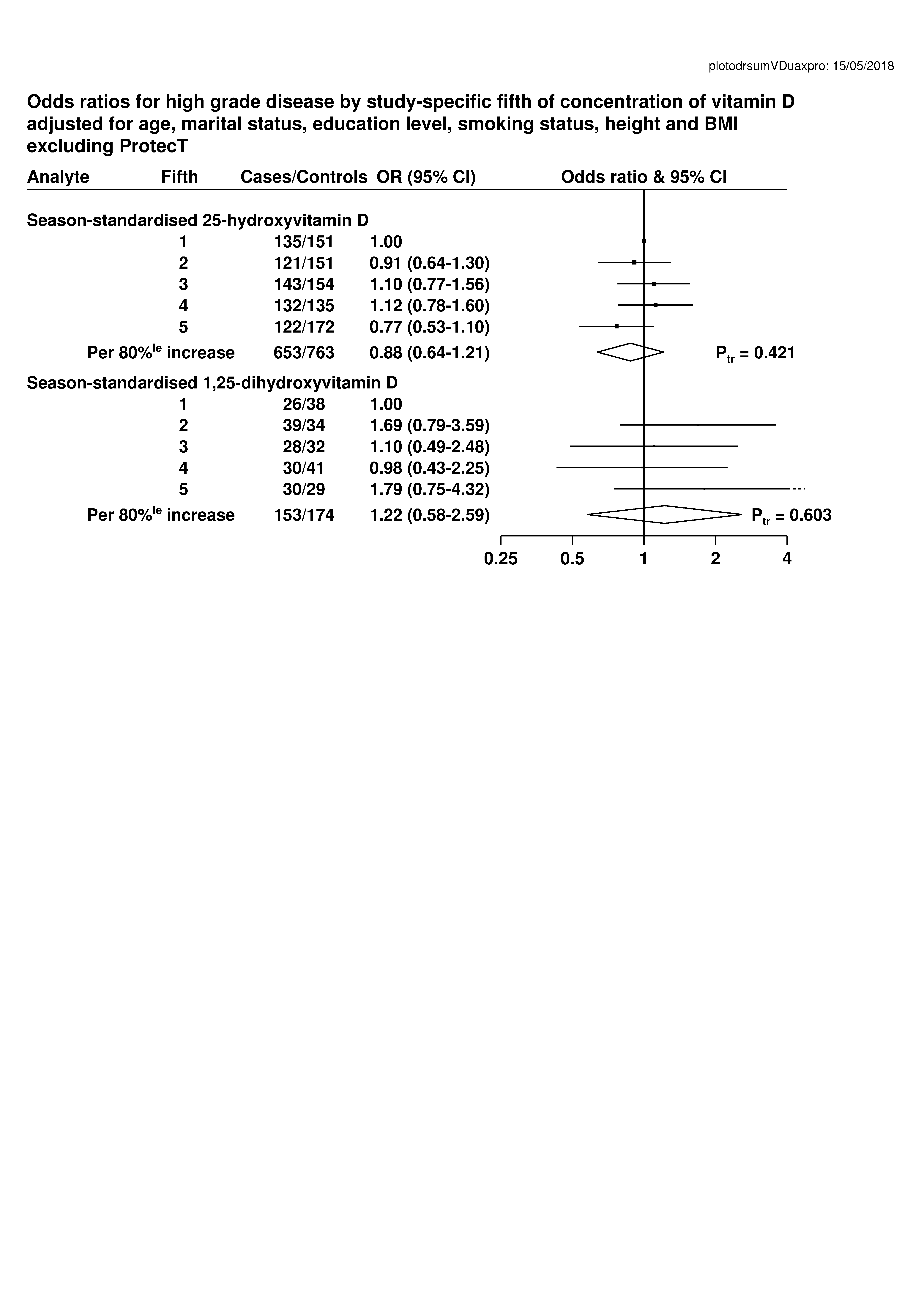
1. **Advanced stage prostate cancer** a

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1. **Aggressive prostate cancer** b

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1. **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.

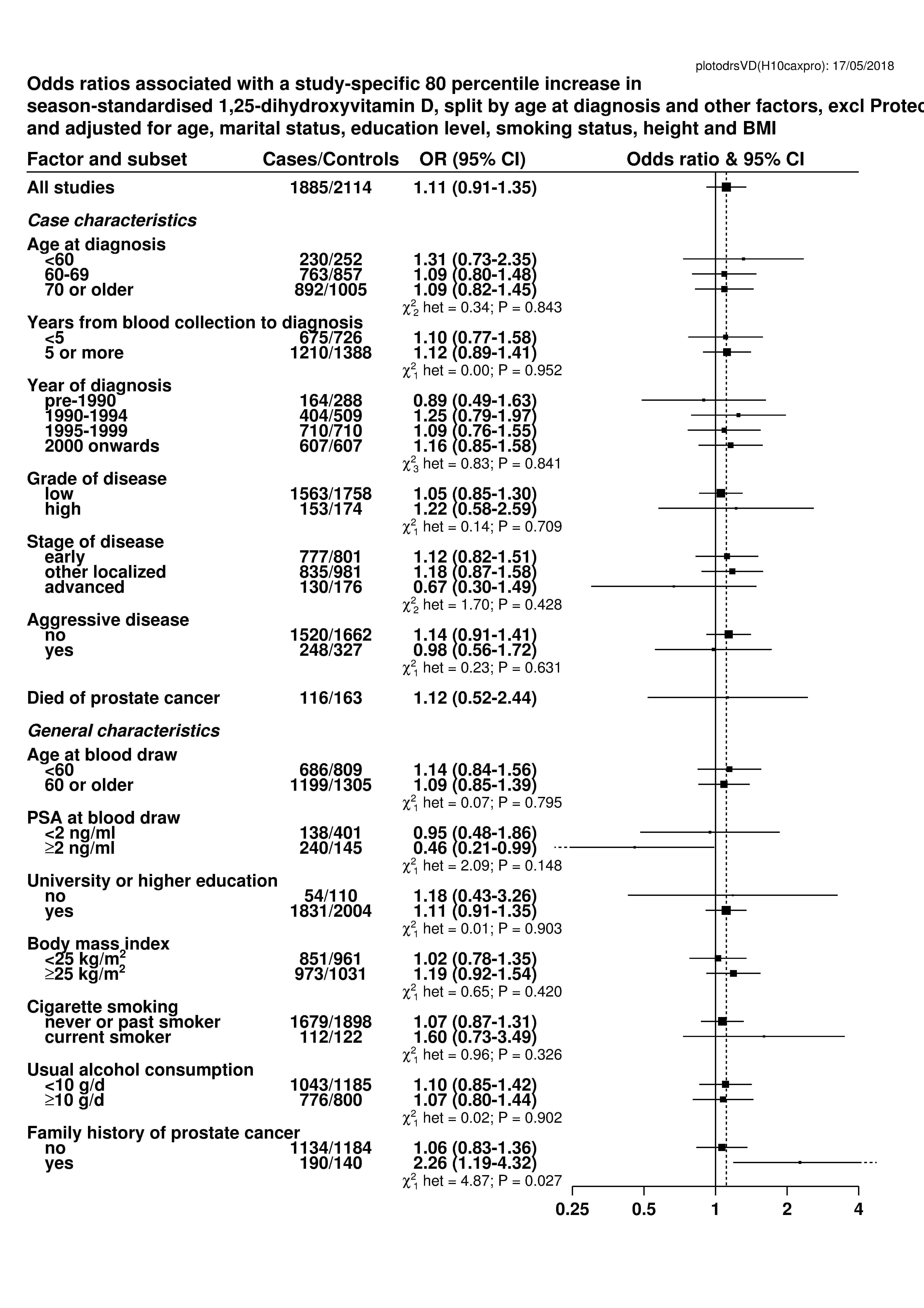
Estimates are from logistic regression conditioned on the matching variables and adjusted for exact age, marital status, education, smoking, height and body mass indexa. Abbreviations: 80%le= 80 percentile; CI = confidence interval; Ptr = *P*trend.

*P*trend (Ptr) 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.

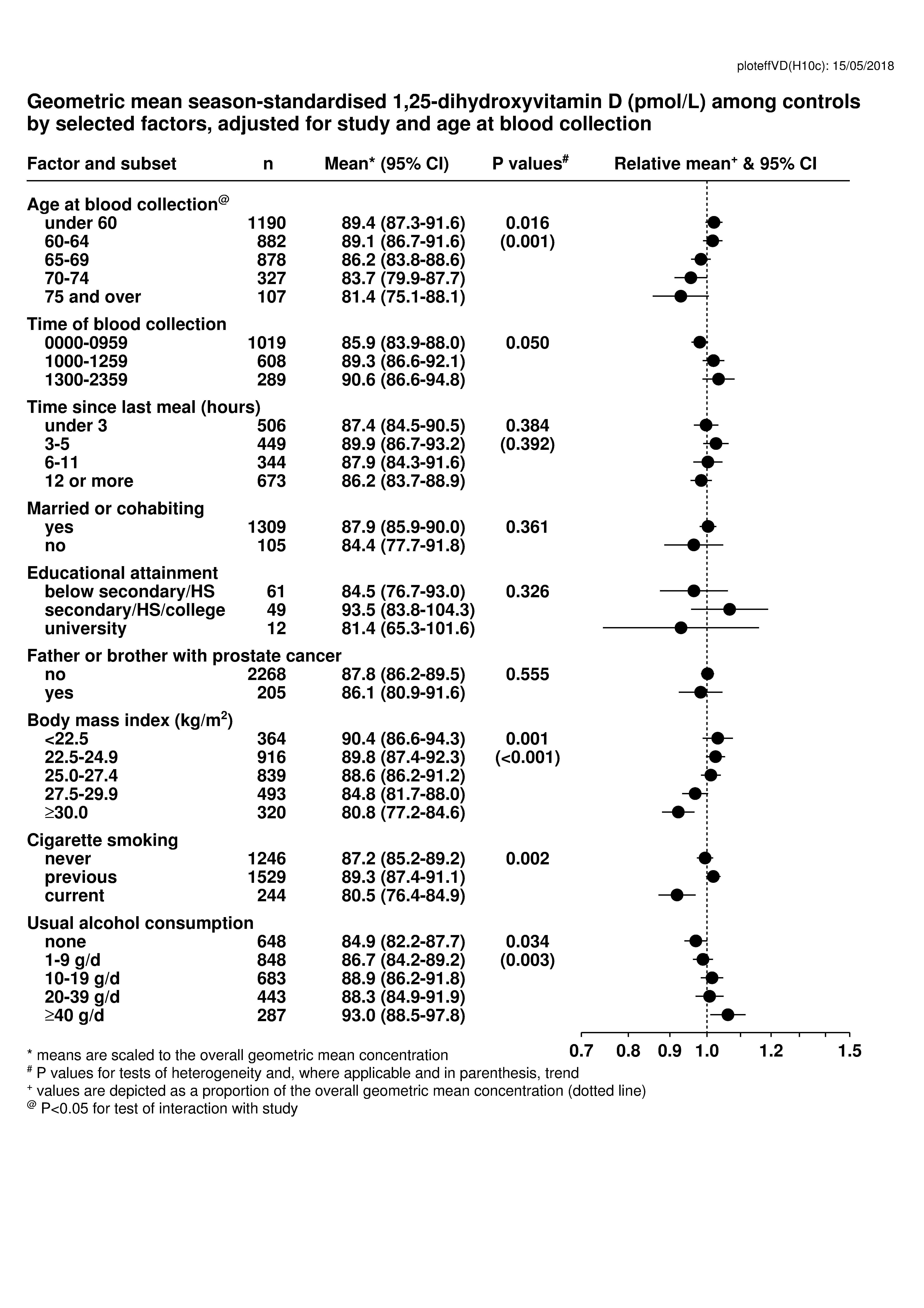
c Prostate cancer was defined as high-grade if the Gleason sum was at least 8 or equivalent (i.e. undifferentiated).

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**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).

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**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.