Journal of Thrombosis and Haemostasis, **11**: 423–431 DOI: 10.1111/jth.12118

ORIGINAL ARTICLE

25-Hydroxyvitamin D concentrations and risk of venous thromboembolism in the general population with 18 791 participants

P. BRØNDUM-JACOBSEN,*†‡ M. BENN,†‡§ A. TYBJÆRG-HANSEN†‡¶** and B. G. NORDESTGAARD*†‡**

**Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital; †The Copenhagen General Population Study, Herlev Hospital, Copenhagen University Hospital; ‡The Faculty of Health and Medical Sciences, University of Copenhagen; §Department of Clinical Biochemistry, Gentofte Hospital, Copenhagen University Hospital; ¶Department of Clinical Biochemistry, Rigshospitalet, Copenhagen University Hospital; and **The Copenhagen City Heart Study, Bispebjerg Hospital, Copenhagen University Hospital, Copenhagen, Denmark*

To cite this article: Brøndum-Jacobsen P, Benn M, Tybjærg-Hansen A, Nordestgaard BG. 25-Hydroxyvitamin D concentrations and risk of venous thromboembolism in the general population with 18 791 participants. *J Thromb Haemost* 2013; **11**: 423–31.

Summary. *Background:* Vitamin D has potential antithrombotic effects, suggesting that vitamin D analogs could be used as adjunctive antithrombotic agents. However, epidemiologic evidence of an association between reduced 25-hydroxyvitamin D concentrations and the risk of venous thromboembolism is lacking. *Objectives:* To test the hypothesis that reduced plasma 25-hydroxyvitamin D concentrations are associated with an increased risk of venous thromboembolism in the general population. *Methods:* We prospectively studied 18 791 participants from the Copenhagen City Heart Study and the Copenhagen General Population Study. During up to 30 years of follow-up, 950 participants were diagnosed with venous thromboembolism. Plasma 25-hydroxyvitamin D concentrations were adjusted for seasonal variation. *Results:* The cumulative incidence of venous thromboembolism as a function of age increased with decreasing tertiles of seasonally adjusted plasma 25-hydroxyvitamin D (log-rank trend: $P = 4 \times 10^{-4}$). On comparison of participants in the lowest and the highest tertile of plasma 25-hydroxyvitamin D concentrations, the crude risk estimate in a model adjusted for age and sex was a 37% (95% confidence interval [CI] 15–64%) increased risk of venous thromboembolism. The corresponding risk increase in a model adjusted for age, sex, body mass index, smoking and cancer was 26% (95% CI 5–51%), and in a multivariable-adjusted model also including physical activity, hormone replacement therapy, menopausal status, oral contraception use and lipid-lowering therapy it was 28% (95% CI 6–53%). Furthermore, corresponding risk increases with attempts to correct for regression dilution bias were 103% (95% CI 37–202%), 70% (95% CI 14–155%) and 73% (95% CI 15–160%) in the three models, respectively. *Conclusion:* In these large general population studies, we observed a stepwise increasing risk of venous thromboembolism with decreasing tertiles of seasonally adjusted plasma 25-hydroxyvitamin D concentrations.

Keywords: 25-hydroxyvitamin D, population study, prospective study, venous thromboembolism, vitamin D, vitamin D deficiency.

Correspondence: Børge G. Nordestgaard, Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark.
Tel.: +45 38683297; fax: +45 38683311.
E-mail: Boerge.Nordestgaard@regionh.dk

Received 27 July 2012, accepted 13 December 2012

Introduction

Venous thromboembolism and atherosclerotic disease may be more closely related than previously thought, e.g. through thrombosis formation [1–3]. Reduced 25-hydroxyvitamin D, which is generally accepted to be the best marker of a vitamin D-deficient state [4], has, in several epidemiologic studies, been associated with an increased risk of atherosclerotic disease in the coronary arteries [5–7], cerebral arteries [8–10], and peripheral arteries [11]. The mechanisms proposed to drive these associations have included reduced 25-hydroxyvitamin D concentrations leading to a prothrombotic state [12]. It has even been suggested that vitamin D analogs could be used as adjunctive antithrombotic agents [13]. However, there is a lack of epidemiologic evidence on the association between reduced 25-hydroxyvitamin D concentrations and the risk of venous thromboembolism.

In the present study, we therefore tested the hypothesis that reduced 25-hydroxyvitamin D concentrations are associated with an increased risk of venous thromboembolism in the general population. We included 18 791 participants from the Copenhagen City Heart Study and the Copenhagen General Population Study with baseline plasma 25-hydroxyvitamin D measurements, and followed them for up to 30 years.

Methods

The studies were approved by Danish ethical committees (No. KF-V.100.2039/91 and H-KF-01-144/01), and conducted according to the Declaration of Helsinki. Written informed consent was obtained from participants.

Study cohorts

The Copenhagen City Heart Study is a prospective study initiated in 1976–1978 with follow-up examinations in 1981–1983, 1991–1994, and 2001–2003 [14]. The Copenhagen General Population Study is a prospective study initiated in 2003, with ongoing enrollment [15]. Both cohorts represented the general population of Copenhagen, Denmark, and all endpoints were ascertained from 1976 to May 2011. Participants were randomly selected from the national Danish Civil Registration System to represent the Danish general population aged 20–100 years from Copenhagen and suburbs. There is no overlap of participants between the two studies. At each examination, participants completed a questionnaire, underwent a physical examination, and provided blood samples. We included a total of 18 791 participants: 10 170 from the 1981–1983 examination of the Copenhagen City Heart Study (participation rate of 56%), and 8621 from the Copenhagen General Population Study examined in 2004–2005 (participation rate of 50%), all without a prior history of venous thromboembolism and with plasma available for 25-hydroxyvitamin D measurement. The mean follow-up time until May 2011 was 13 years (range: 0–30 years). All participants were followed from baseline in 1981–1983 or in 2004–2005 until the occurrence of venous thromboembolism ($n = 950$), death ($n = 7171$), emigration ($n = 99$), or May 2011, whichever came first. Follow-up was 100% complete; that is, we did not lose track of even a single individual.

Endpoint

Without knowledge of 25-hydroxyvitamin D concentrations, information on diagnoses of deep vein thrombosis (World Health Organization; International Classification of Diseases, 8th edition, codes 451.00, 451.08–09, 451.90, 451.92, 671.01–03, and 671.08–09, and 10th edition, codes I80.1–3, 022.3, and 087.1) and pulmonary embolism (8th edition, codes 450.99 and 973.99, and 10th edition, codes I.26.0, I26.9, and 0.88.2) were obtained from the national Danish Patient Registry and the national Danish Causes of Death Registry. Participants with either deep vein thrombosis, pulmonary embolism or both were coded as having venous thromboembolism, as done previously [16–18]. The diagnostic criteria used were ultrasonography or venography in the case of deep vein thrombosis, and ventilation/perfusion scintigraphy, ventilation/perfusion computed tomography and/or computerized tomographic pulmonary angiography in the case of pulmonary embolism. Register-based diagnoses of venous thromboembolism in Denmark have previously been validated, with an overall positive predictive value of a discharge diagnosis of venous thromboembolism of 55–72% [16,19].

Biochemical analysis

Plasma 25-hydroxyvitamin D was measured without knowledge of venous thromboembolism, by use of a competitive chemiluminescence immunoassay (DiaSorin, Stillwater, MN, USA), with intra-assay and inter-assay coefficients of variation of 10% and 11% for controls with a concentration of ~ 40 nmol L^{-1}, and a lower detection limit of 10 nmol L^{-1}; 1.7% of samples were below this limit, and were assigned a concentration of 7.5 nmol L^{-1} for statistical analyses. Plasma 25-hydroxyvitamin D concentrations were measured during January 2010 to April 2012, using samples stored at − 20 °C (Copenhagen City Heart Study) or − 80 °C (Copenhagen General Population Study) without previous thawing or exposure to sunlight; values were not normalized to fresh control samples; however, our mean levels of 45 nmol L^{-1} were similar to those measured by others [9,20].

Other covariates

Analyses were adjusted for covariates known to influence 25-hydroxyvitamin D levels or the risk of venous thromboembolism. Body mass index was expressed as measured weight in kilograms divided by measured height in meters squared, and was used on a continuous scale for adjustment, and coded categorically as low (< 25 kg m^{-2}) or high (≥ 25 kg m^{-2}) for stratification. Smoking status was classified categorically as never, current or former smokers, or on a continuous scale as packyears smoked. Information on any cancer diagnosis was obtained from the national Danish Cancer Registry, which captures 98% of all cancers in Denmark [21]. Physical activity for adjustments was classified as physical activity during work (five categories) and during leisure time (four categories), and for stratification was coded in one variable as low or high; low physical activity was predominantly sedentary work and < 2 h of leisure time physical activity per week, and high physical activity was predominantly heavy manual work and/or > 2 h of leisure time physical activity per week. Hormone replacement therapy, meno-

pausal status, use of oral contraception and lipid-lowering therapy were all self-reported.

Statistical analyses

Data were analyzed with STATA/SE (StataCorp, College Station, Texas, USA). To adjust for seasonal variation in plasma 25-hydroxyvitamin D levels, we assigned participants to percentiles of plasma 25-hydroxyvitamin D concentration by month of sample collection; this has been shown to be a valid method for adjustment for seasonal variability in a biomarker when only a single sample is available [22]. Participants were grouped according to plasma 25-hydroxyvitamin D levels into tertiles, with the highest tertile as a reference group.

We plotted cumulative incidence of venous thromboembolism against age with the Kaplan–Meier method, and used log-rank trend tests across seasonally adjusted tertile groups of plasma 25-hydroxyvitamin D concentrations (coded as 1, 2, and 3). For test of trend in Cox regression models (see below), we likewise categorized tertile groups coded as 1, 2, and 3; we tested for linearity by including a squared 25-hydroxyvitamin D tertile group, and found no evidence of non-linearity.

We used Cox proportional hazard regression models with age as a time scale, implying that age is automatically adjusted for, and the use of left truncation (delayed entry) to examine the association between seasonally adjusted tertile groups of plasma 25-hydroxyvitamin D concentrations and risk of venous thromboembolism; left truncation means that participants only entered into the Cox model at their age at examination in 1981–1983 or 2004–2005, when blood for 25-hydroxyvitamin D measurement was drawn. Multivariable Cox regression analyses included in one model adjustment for age, sex, body mass index, smoking, and cancer, and in another model adjustment for age, sex, body mass index, smoking, cancer, physical activity, hormone replacement therapy, menopausal status, oral contraception use, and lipid-lowering therapy. For adjustments, age and body mass index were on a continuous scale, smoking was in three groups (current, former, and never), physical activity was in nine categories, and the remaining covariates were dichotomized. We tested the assumption of proportional hazards graphically by plotting – ln (survival probability) against ln (analysis time). Suspicion of non-parallel lines was further tested by using Schoenfeld residuals; however, we did not detect major violations of the proportional hazard assumption. Missing values in the body mass index (n = 126), smoking (n = 154), physical activity (n = 312) and menopausal status covariates (n = 9) were imputed by using multivariable normal regression imputation, where age at baseline and sex were independent variables in the model. Hazard ratios including 95% confidence intervals (CIs) were corrected for regression dilution bias with a non-parametric method [23]; this correction influences the magnitude of the hazard ratios and CIs, but does not affect the significance level or the direction of the association. We used 25-hydroxyvitamin D values from 400 individuals without chronic diseases who attended both the baseline 1981–1983 examination and the 2001–2003 examination of the Copenhagen City Heart Study, to compute a regression dilution ratio of 0.45.

Interaction between tertile groups of seasonally adjusted plasma 25-hydroxyvitamin D concentrations and venous thromboembolism was evaluated by including two-factor interaction terms, one at a time, in the multivariable Cox regression model.

Results

Table 1 and Table S1 show baseline characteristics of the 18 791 participants from the Copenhagen City Heart Study (n = 10 170) and the Copenhagen General Population Study (n = 8621), both representing the Danish general population. During follow-up, 950 venous thromboembolic events occurred. Seasonal variation in venous thromboembolic events and plasma 25-hydroxyvitamin D concentrations are shown in Fig. 1: there was no specific pattern in the seasonal variation for venous thromboembolism, whereas, for 25-hydroxyvitamin D concentrations, we observed the lowest concentrations in February and the highest in September.

The cumulative incidence of venous thromboembolism as a function of age increased with decreasing tertiles of seasonally adjusted plasma 25-hydroxyvitamin D concentrations (log-rank trend, $P = 4 \times 10^{-4}$) (Fig. 2).

Decreasing tertiles of seasonally adjusted plasma 25-hydroxyvitamin D concentrations were associated with a stepwise increasing risk of venous thromboembolism (Fig. 3, left column: P for trend, 5×10^{-4} to 9×10^{-3}). On comparison of participants with plasma 25-hydroxyvitamin D concentrations in the lowest tertile with participants with plasma 25-hydroxyvitamin D concentrations in the highest tertile, the crude risk estimate in a model adjusted for age and sex was a 37% (95% CI 15–64%) increased risk of venous thromboembolism. The corresponding risk increase in a model adjusted for age, sex, body mass index, smoking and cancer was 26% (95% CI 5–51%), and in a multivariable-adjusted model also including physical activity, hormone replacement therapy, menopausal status, oral contraception use and lipid-lowering therapy it was 28% (95% CI 6–53%) (Fig. 3). Furthermore, the corresponding risk increases with attempts to correct for regression dilution bias were 103% (95% CI 37–202%) in the model adjusted for age and sex, 70% (95% CI 14–155%) in the model adjusted for age, sex, body mass index, smoking, and cancer, and 73% (95% CI 15–160%) in the multivariable-adjusted model (Fig. 3).

In a sensitivity analysis restricted to events occurring between 1995 and 2011, with potentially more reliable

Table 1 Baseline characteristics of participants from the Copenhagen City Heart Study and the Copenhagen General Population Study

Characteristics	The Copenhagen City Heart Study	The Copenhagen General Population Study	Overall	Missing information, *N* (%)
Number of participants	10 170	8621	18 791	
25-Hydroxyvitamin D (nmol L^{-1})	44 (26–58)	46 (30–59)	45 (28–59)	0 (0)
Age (years)	57 (49–65)	57 (47–66)	57 (48–65)	0 (0)
Women (%)	56	53	55	0 (0)
Venous thromboembolism*	653	297	950	0 (0)
Deep vein thrombosis*	364	183	547	0 (0)
Pulmonary embolism*	289	114	403	0 (0)
Body mass index (kg m^{-2})	25 (22–28)	26 (23–28)	26 (23–28)	126 (0.7)
Current smoking (%)	58	27	43	154 (0.8)
Cancer* (%)	33	16	25	0 (0)
High physical activity† (%)	66	45	58	312 (1.7)
Hormone replacement therapy‡ (%)	16	7	10	0 (0)
Menopause‡ (%)	42	34	38	9 (0.05)
Oral contraception use‡ (%)	4	3	4	0 (0)
Lipid-lowering therapy (%)	0	8	3	0 (0)

Continuous variables are reported as median and interquartile range, and categorical variables are reported as percentages or as numbers for the endpoints. Data are from the 1981–1983 examination of the Copenhagen City Heart Study and from the Copenhagen General Population Study on participants examined in 2004–2005. Conversion factors: to convert plasma 25-hydroxyvitamin D in nmol L^{-1} to ng mL^{-1}, divide by 2.496. *Number of events at the end of follow-up; some participants with pulmonary embolism also had deep vein thrombosis. †For details on physical activity, see Table S1. ‡Women only.

diagnosis of venous thromboembolism, the multivariable and regression dilution bias adjusted risk for lowest vs. highest tertile of 25-hydroxyvitamin D was increased by 176% (95% CI 42–436%) (Fig. 3, right panels). In a further sensitivity analysis with follow-up time restricted to the first 5 years, and thus with substantially less statistical power and possibly less reliable diagnosis of venous thromboembolism, corresponding risk estimates were similar in magnitude to the overall risk estimates, but insignificant for all three models (Fig. S1). Finally, in a sensitivity analysis using a smoking variable on a continuous scale (packyears smoked) in the multivariable-adjusted models instead of a categorical smoking variable, risk estimates were more extreme than in the previously reported multivariable adjusted models (Fig. S2).

In stratified analyses, participants in the lowest tertile of seasonally adjusted 25-hydroxyvitamin D concentrations had an increased risk of venous thromboembolism as compared with those in the highest tertile in some individual strata; however, in several strata, the individual risk estimates did not reach statistical significance (Fig. 4). Nevertheless, for all covariates, tests of interaction between tertiles of plasma 25-hydroxyvitamin D concentrations and covariates on risk of venous thromboembolism did not show any evidence of interaction (Fig. 4), implying that low 25-hydroxyvitamin D concentrations are associated with an increased risk of future venous thromboembolism irrespective of the presence or absence of the potential confounders studied. Notably, however, the risk of venous thromboembolism in smokers with the lowest vs. highest tertile of seasonally adjusted 25-hydroxyvitamin D concentrations was increased by 113% (95% CI 24–264%), whereas the corresponding value in non-smokers was − 4% (95% CI − 50% to 82%).

Discussion

In this large general population study including 18 791 participants, we observed an increasing risk of venous thromboembolism with decreasing tertiles of seasonally adjusted plasma 25-hydroxyvitamin D concentrations. There are no previous similar studies. Although these results are robust, they should not be taken to infer causality.

Mechanistically, the present findings can be explained by several lines of evidence suggesting an important physiologic role of the vitamin D system in the maintenance of antithrombotic homeostasis. First, in vitamin D receptor knockout mice mimicking a vitamin D-deficient state, there is significant downregulation of the gene expression of antithrombin in the liver and of thrombomodulin in the aorta, and upregulation of the gene expression of tissue factor in the liver and kidneys, overall leading to a prothrombotic state; indeed, the development of exacerbated multiorgan thrombus formation upon exogenous lipopolysaccharide injection is observed in this model [24]. Also, in human aortic smooth muscle cells, vitamin D analogs have been shown to modulate the expression of plasminogen activator inhibitor-1, thrombospondin-1, and thrombomodulin, leading to an overall antithrombotic effect [25]. As the natures of atherosclerotic disease and venous thromboembolism may not be as separate as previously thought [2], and as the two diseases share some common risk factors [1,26], it may be

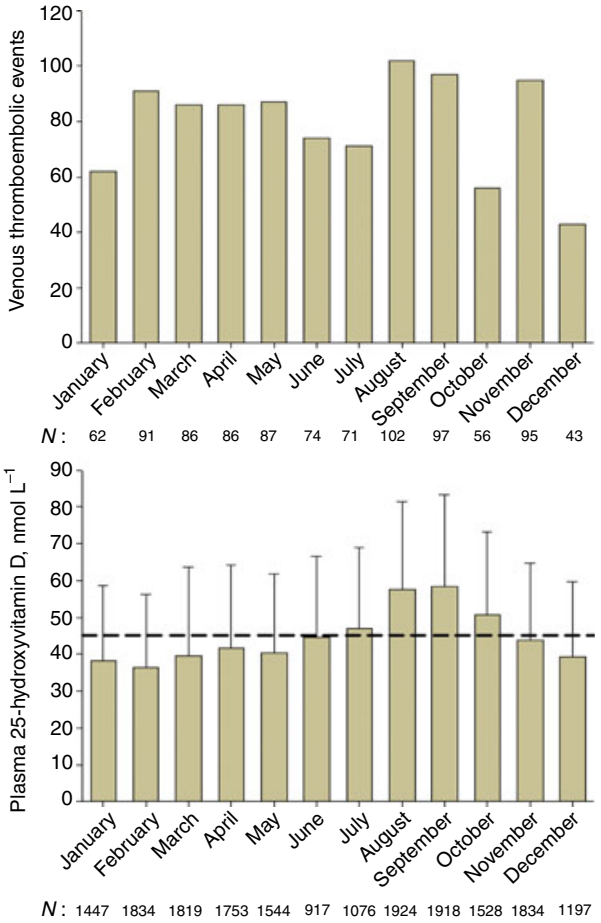

Fig. 1. Seasonal variation in incident cases of venous thromboembolism and mean plasma 25-hydroxyvitamin D concentrations as a function of sampling month in 18 791 participants from the Danish general population, the Copenhagen City Heart Study and the Copenhagen General Population Study. Whiskers represent plus one standard deviation. The dashed line represents the overall mean of plasma 25-hydroxyvitamin D concentrations of 45 nmol L^{-1}.

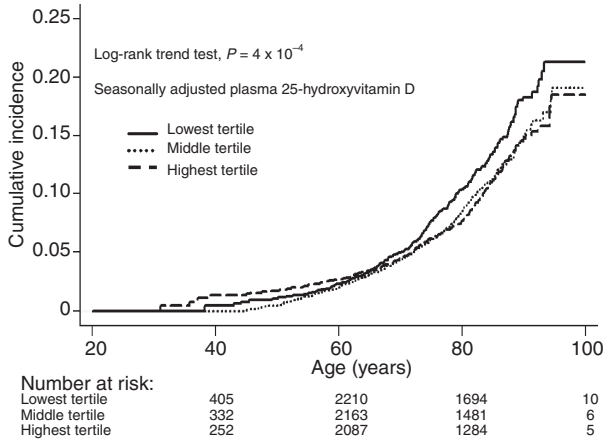

Fig. 2. Cumulative incidence by the Kaplan–Meier method of venous thromboembolism as a function of age and by tertile group of seasonally adjusted plasma 25-hydroxyvitamin D concentrations. Log-rank trend tests are for trend across tertile groups coded as 1, 2, and 3.

hypothesized that reduced plasma 25-hydroxyvitamin D concentration could be yet another common risk factor. Nevertheless, it is also a possibility that a reduced 25-hydroxyvitamin D concentration is merely a marker of other common risk factors, such as smoking, obesity, and old age. Indeed, we observed that smokers in the lowest vs. highest tertile of seasonally adjusted 25-hydroxyvitamin D concentrations had an increased risk of venous thromboembolism, whereas this was not the case in nonsmokers. This makes biological sense, as smoking is a risk factor for venous thromboembolism, and is also associated with reduced 25-hydroyxvitamin D concentrations; however, in the present study, we did not find an indication of confounding of the association between 25-hydroxyvitamin D concentrations and the risk of venous thromboembolism by heavy smoking when adjusting multivariably, including a smoking variable on a continuous scale.

The strengths of the present study include the two large prospective samples from a homogeneous general population. Other strengths include the detailed information on several potential confounders, a high participation rate, an attempt to correct for regression dilution bias, a long follow-up time with no loss to follow-up, a high number of events, the use of registry-based diagnoses without knowledge of plasma 25-hydroxyvitamin D concentrations and measurement of plasma 25-hydroxyvitamin D without knowledge of diagnoses of venous thromboembolism. Finally, it is a strength that we assigned participants into 25-hydroxyvitamin D tertiles, thus eliminating the seasonal variation in 25-hydroxyvitamin D concentrations. Seasonal variability in incident cases of venous thromboembolism has previously been described, with an increased incidence of venous thromboembolism in winter [27]. In the present study, we did not find such seasonal variation, in agreement with another study [28]; however, the lack of seasonal variation in these studies (including 950 and 2666 cases, respectively) could be attributable to them having less statistical power than the above mentioned meta-analysis including 23 469 cases [27].

Potential limitations include the fact that some degradation of plasma 25-hydroxyvitamin D may have occurred during storage at − 20 °C in the samples from the Copenhagen City Heart Study; however, such a potential degradation is unlikely to have affected the results of the present study to a large extent, because 25-hydroxyvitamin D has been observed to be minimally degradable during storage at − 20 °C [29]. Furthermore, samples from the Copenhagen General Population Study were stored at − 80 °C, and there was no difference in risk estimates between the two studies. Also, the mean 25-hydroxyvitamin D concentration in both studies was similar, at 45 nmol L^{-1}. Another potential limitation is

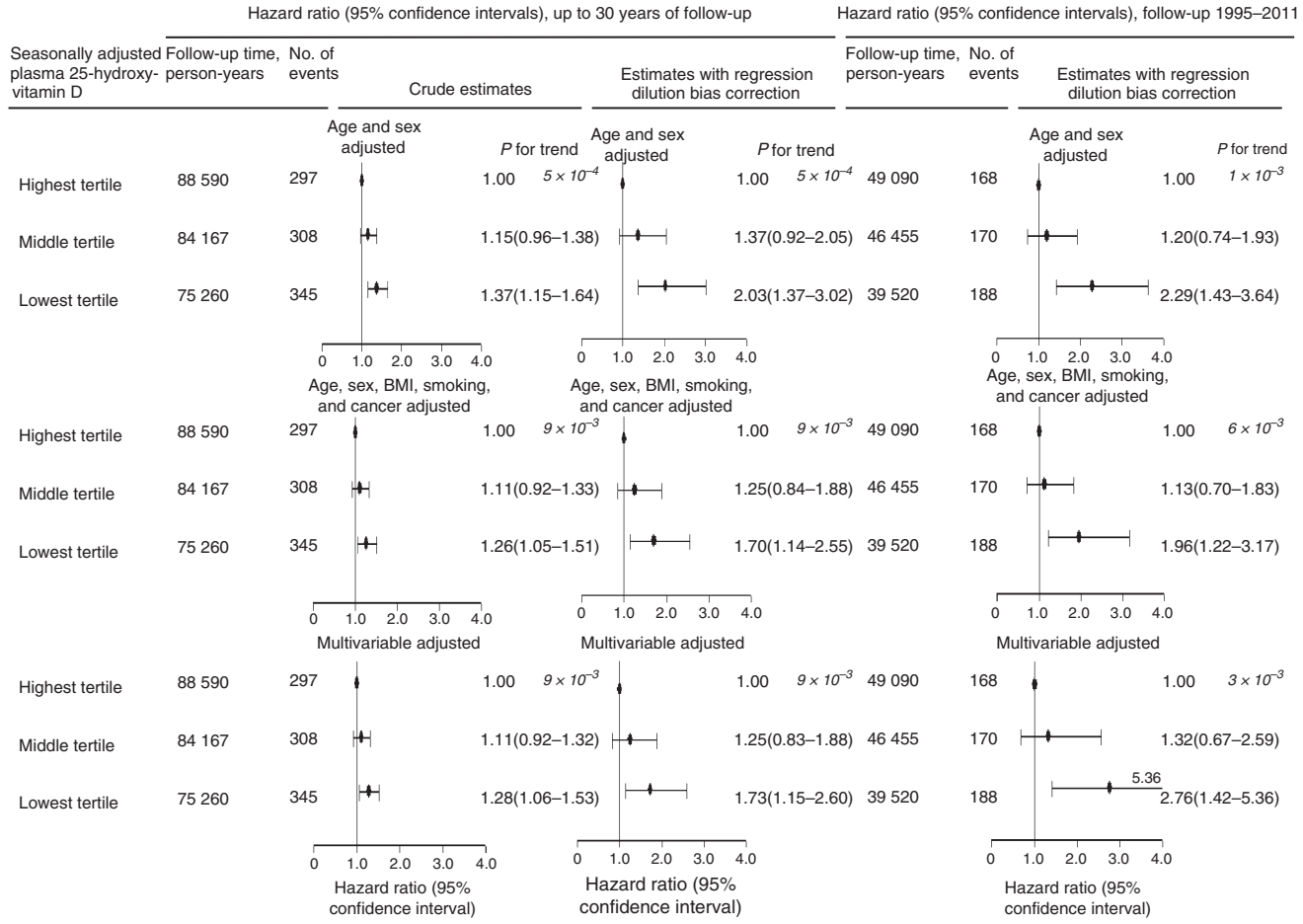

Fig. 3. Risk of venous thromboembolism as a function of seasonally adjusted tertile groups of plasma 25-hydroxyvitamin D concentration. The left panels show results in the entire follow-up period (reported as both crude estimates and with attempts to correct for regression dilution bias), and in the follow-up period 1995–2011 (only regression dilution bias corrected estimates reported). Multivariable adjustment was for age, sex, body mass index (BMI), smoking, cancer, physical activity, hormone replacement therapy, menopausal status, oral contraception use, and lipid-lowering therapy. Black dots represent hazard ratios, and error bars the 95% confidence intervals. *P*-values for trend test across tertiles coded as 1, 2 and 3 were estimated with Cuzick's extension of a Wilcoxon rank-sum test.

selection bias, which is also unlikely to have affected the results in the present study to a large degree, as participants were randomly selected from the general population in both studies. Yet another potential limitation is misclassification of venous thromboembolism diagnoses, as some of these were based on clinical suspicion only, and as objective methods, such as ultrasound and computed tomography, were not widely available in the 1980s; however, such misclassification would probably be non-differential, and therefore only bias the results toward the null hypothesis. An indication of a lower degree of non-differential misclassification with time in our study is that the risk estimates of a sensitivity analysis with follow-up time restricted to the period 1995–2011 are more extreme than the risk estimates of the total follow-up period. This could be explained by more reliable diagnosis of venous thromboembolism over time, and therefore the true association between low 25-hydroxyvitamin D concentrations and the risk of venous thromboembolism may be more pronounced than the results of the total follow-up period indicate. In addition, as covariates for adjustment were only obtained at baseline, residual confounding could be present. Also, by using regression dilution bias based on samples taken 20 years apart, we may have overcorrected the magnitude of the hazard ratios [23]; however, we also provide the crude hazard ratios, and the real effect sizes are probably in between the crude and regression dilution corrected hazard ratios. Yet another limitation is that the diagnoses of venous thromboembolism are register-based and not validated individually. Finally, as plasma 25-hydroxyvitamin D concentrations differ between people with different skin colors [30], and depend on the amount of sun exposure, our results may not necessarily apply to all people, but certainly would be applicable to other populations with similar skin color and living in countries with similar sun exposure as in northern Europe.

In conclusion, we observed a stepwise increasing risk of venous thromboembolism with decreasing tertiles of

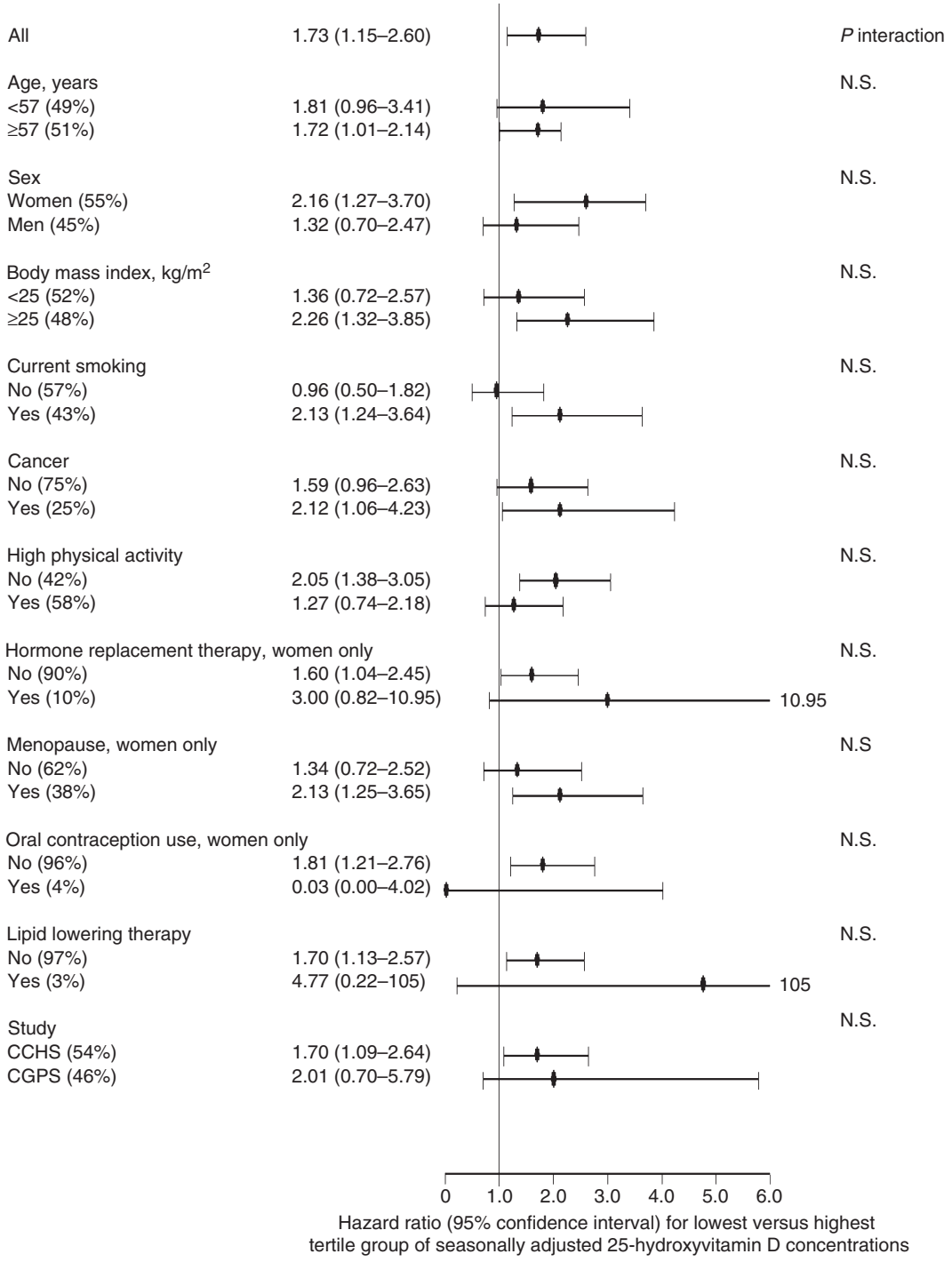

Fig. 4. Risk of venous thromboembolism for the lowest vs. the highest tertile group of seasonally adjusted plasma 25-hydroxyvitamin D concentrations. Black diamonds represent hazard ratios, and error bars the 95% confidence intervals. Hazard ratios were adjusted multivariably for age, sex, body mass index, smoking, cancer, physical activity, hormone replacement therapy, menopausal status, oral contraception use, and lipid-lowering therapy. *P*-values are for the test of interaction between plasma 25-hydroxyvitamin D in tertiles and covariates with Bonferroni correction: *P*-values were multiplied by 10 for the number of individual tests performed. NS, not significant, as the original *P*-value multiplied by 10 to account for multiple comparison was > 1.0. CCHS, Copenhagen City Heart Study; CGPS, Copenhagen General Population Study.

seasonally adjusted plasma 25-hydroxyvitamin D concentrations. The consistent and stepwise increasing risk may tempt us to suggest causality; however, one can not infer causality from the present study. Therefore, randomized intervention trials or Mendelian randomization studies are needed to test the question of causality, and randomized

trials with vitamin D supplementation are needed before supplementation is implemented in the general population or in selected patient groups to reduce the risk of venous thromboembolism.

Acknowledgements

We are indebted to the staff and participants of the Copenhagen City Heart Study and of the Copenhagen General Population Study for their important contributions. We thank R. Aamo, A. Teo Hansen and A. Pedersen for measurement of plasma 25-hydroxyvitamin D.

Disclosure of Conflict of Interests

The study was funded by the Danish Heart Foundation, the Faculty of Health Sciences, University of Copenhagen, and by Herlev Hospital, Copenhagen University Hospital. These are public or non-profit private sources with no right to approve or disapprove of the present results. DiaSorin provided free kits for measurement of plasma 25-hydroxyvitamin D, but had no influence on the submitted work and had no right to approve or disapprove of the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Risk of venous thromboembolism as a function of seasonally adjusted tertile groups of plasma 25-hydroxyvitamin D concentration with follow-up time restricted to the first 5 years, reported both as crude estimates and as estimates with correction for regression dilution bias.

Figure S2. Risk of venous thromboembolism as a function of seasonally adjusted tertile groups of plasma 25-hydroxyvitamin D, reported both as crude estimates and as estimates with correction for regression dilution bias in the entire follow-up period.

Table S1. Physical activity characteristics for participants in the Danish general population, the Copenhagen City Heart Study and the Copenhagen General Population Study.

References

1 Holst AG, Jensen G, Prescott E. Risk factors for venous thromboembolism: results from the Copenhagen City Heart Study. *Circulation* 2010; **121**: 1896–903.
2 Prandoni P. Venous thromboembolism and atherosclerosis: is there a link? *J Thromb Haemost* 2007; **5** (Suppl. 1): 270–5.
3 Prandoni P, Bilora F, Marchiori A, Bernardi E, Petrobelli F, Lensing AW, Prins MH, Girolami A. An association between atherosclerosis and venous thrombosis. *N Engl J Med* 2003; **348**: 1435–41.
4 Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* 2009; **19**: 73–8.
5 Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008; **117**: 503–11.
6 Giovannucci E. Vitamin D and cardiovascular disease. *Curr Atheroscler Rep* 2009; **11**: 456–61.
7 Brondum-Jacobsen P, Benn M, Jensen GB, Nordestgaard BG. 25-Hydroxyvitamin D levels and risk of ischemic heart disease myocardial infarction, and early death: population-based study and meta-analyses of 18 and 17 studies. *Arterioscler Thromb Vasc Biol* 2012; **32**: 2794–802.
8 Pilz S, Dobnig H, Fischer JE, Wellnitz B, Seelhorst U, Boehm BO, März W. Low vitamin D levels predict stroke in patients referred to coronary angiography. *Stroke* 2008; **39**: 2611–13.
9 Kilkkinen A, Knekt P, Aro A, Rissanen H, Marniemi J, Heliovaara M, Impivaara O, Reunanen A. Vitamin D status and the risk of cardiovascular disease death. *Am J Epidemiol* 2009; **170**: 1032–9.
10 Broendum-Jacobsen P, Nordestgaard BG, Schnohr P, Marianne B. 25-Hydroxyvitamin D and risk of symptomatic stroke: an original study and meta-analysis. *Ann Neurol* 2012, DOI: 10.1002/ana23738
11 Melamed ML, Muntner P, Michos ED, Uribarri J, Weber C, Sharma J, Raggi P. Serum 25-hydroxyvitamin D levels and the prevalence of peripheral arterial disease: results from NHANES 2001–2004. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1179–85.
12 Ohsawa M, Koyama T, Yamamoto K, Hirosawa S, Kamei S, Kamiyama R. 1-alpha,25-dihydroxyvitamin D(3) and its potent synthetic analogs downregulate tissue factor and upregulate thrombomodulin expression in monocytic cells, counteracting the effects of tumor necrosis factor oxidized LDL. *Circulation* 2000; **102**: 2867–72.
13 Koyama T, Shibakura M, Ohsawa M, Kamiyama R, Hirosawa S. Anticoagulant effects of 1alpha,25-dihydroxyvitamin D3 on human myelogenous leukemia cells and monocytes. *Blood* 1998; **92**: 160–7.
14 Langsted A, Freiberg JJ, Tybjaerg-Hansen A, Schnohr P, Jensen GB, Nordestgaard BG. Nonfasting cholesterol and triglycerides and association with risk of myocardial infarction and total mortality: the Copenhagen City Heart Study with 31 years of follow-up. *J Intern Med* 2011; **270**: 65–75.
15 Klovaite J, Benn M, Yazdanyar S, Nordestgaard BG. High platelet volume and increased risk of myocardial infarction: 39 531 participants from the general population. *J Thromb Haemost* 2011; **9**: 49–56.
16 Juul K, Tybjaerg-Hansen A, Schnohr P, Nordestgaard BG. Factor V Leiden and the risk for venous thromboembolism in the adult Danish population. *Ann Intern Med* 2004; **140**: 330–7.
17 Frederiksen J, Juul K, Grande P, Jensen GB, Schroeder TV, Tybjaerg-Hansen A, Nordestgaard BG. Methylenetetrahydrofolate reductase polymorphism (C677T), hyperhomocysteinemia, and risk of ischemic cardiovascular disease and venous thromboembolism: prospective and case-control studies from the Copenhagen City Heart Study. *Blood* 2004; **104**: 3046–51.
18 Sode BF, Dahl M, Nielsen SF, Nordestgaard BG. Venous thromboembolism and risk of idiopathic interstitial pneumonia: a nationwide study. *Am J Respir Crit Care Med* 2010; **181**: 1085–92.
19 Severinsen MT, Kristensen SR, Overvad K, Dethlefsen C, Tjønneland A, Johnsen SP. Venous thromboembolism discharge diagnoses in the Danish National Patient Registry should be used with caution. *J Clin Epidemiol* 2010; **63**: 223–8.
20 Semba RD, Houston DK, Bandinelli S, Sun K, Cherubini A, Cappola AR, Guralnik JM, Ferrucci L. Relationship of 25-hydroxyvitamin D with all-cause and cardiovascular disease

mortality in older community-dwelling adults. *Eur J Clin Nutr* 2010; **64**: 203–9.
21 Nielsen SF, Nordestgaard BG, Bojesen SE. Associations between first and second primary cancers: a population-based study. *CMAJ* 2012; **184**: E57–69.
22 Wang Y, Jacobs EJ, McCullough ML, Rodriguez C, Thun MJ, Calle EE, Flanders WD. Comparing methods for accounting for seasonal variability in a biomarker when only a single sample is available: insights from simulations based on serum 25-hydroxyvitamin D. *Am J Epidemiol* 2009; **170**: 88–94.
23 Clarke R, Shipley M, Lewington S, Youngman L, Collins R, Marmot M, Peto R. Underestimation of risk associations due to regression dilution in long-term follow-up of prospective studies. *Am J Epidemiol* 1999; **150**: 341–53.
24 Aihara K, Azuma H, Akaike M, Ikeda Y, Yamashita M, Sudo T, Hayashi H, Yamada Y, Endoh F, Fujimura M, Yoshida T, Yamaguchi H, Hashizume S, Kato M, Yoshimura K, Yamamoto Y, Kato S, Matsumoto T. Disruption of nuclear vitamin D receptor gene causes enhanced thrombogenicity in mice. *J Biol Chem* 2004; **279**: 35798–802.
25 Wu-Wong JR, Nakane M, Ma J. Vitamin D analogs modulate the expression of plasminogen activator inhibitor-1, thrombospondin-1 and thrombomodulin in human aortic smooth muscle cells. *J Vasc Res* 2007; **44**: 11–18.
26 Ageno W, Becattini C, Brighton T, Selby R, Kamphuisen PW. Cardiovascular risk factors and venous thromboembolism: a meta-analysis. *Circulation* 2008; **117**: 93–102.
27 Dentali F, Ageno W, Rancan E, Donati AV, Galli L, Squizzato A, Venco A, Mannucci PM, Manfredini R. Seasonal and monthly variability in the incidence of venous thromboembolism. A systematic review and a meta-analysis of the literature. *Thromb Haemost* 2011; **106**: 439–47.
28 Ribeiro DD, Bucciarelli P, Braekkan SK, Lijfering WM, Passamonti SM, Brodin EE, Rosendaal FR, Martinelli I, Hansen JB. Seasonal variation of venous thrombosis: a consecutive case series within studies from Leiden, Milan and Tromso. *J Thromb Haemost* 2012; **10**: 1704–7.
29 Ocke MC, Schrijver J, Obermann-de Boer GL, Bloemberg BP, Haenen GR, Kromhout D. Stability of blood (pro)vitamins during 4 years of storage at −20 °C: consequences for epidemiologic research. *J Clin Epidemiol* 1995; **48**: 1077–85.
30 Bodnar LM, Catov JM, Wisner KL, Klebanoff MA. Racial and seasonal differences in 25-hydroxyvitamin D detected in maternal sera frozen for over 40 years. *Br J Nutr* 2009; **101**: 278–84.