

## Original article

## Circulating 25-hydroxyvitamin D level and risk of developing rheumatoid arthritis

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## Abstract

**Objective.** The aim of this study was to examine the relationship between preclinical circulating 25-hydroxyvitamin D [25(OH)D] and RA in two nested case-control studies within the prospective cohort Nurses' Health Study (NHS) and NHS II (NHSII).

**Methods.** We included 166 women with RA and blood specimens collected 3 months to 16 years prior to the first RA symptom and 490 matched controls (3:1, matched on age, date of blood draw, hormonal factors). We calculated the odds ratio (OR) and 95% CI for incident RA using conditional logistic regression multivariable adjusted models, including additional covariates for smoking status, parity and breast-feeding, alcohol consumption, BMI, median income and region of residence in the USA. We repeated analyses stratified by time from blood draw to RA diagnosis (3 months to <4 years or ≥4 years) and meta-analysed estimates from the two cohorts using fixed effects models.

**Results.** Incident RA was confirmed in 120 NHS [mean age 63.8 years (s.d. 8.2)] and 46 NHSII participants [mean age 48.5 years (s.d. 4.7)]. Mean time from blood draw to RA diagnosis was 7.8 years (s.d. 4.2) for NHS and 4.2 years (s.d. 2.0) for NHSII participants. Meta-analysis of crude and multivariable-adjusted conditional logistic models did not show significant associations between circulating 25(OH)D and RA. However, among NHSII women with blood drawn between 3 months and <4 years prior to RA diagnosis, there was a 20% decreased risk of RA associated with each 1 ng/ml increase in 25(OH)D [OR 0.80 (95% CI 0.64, 0.99)].

**Conclusion.** We did not observe a significant association between circulating 25(OH)D levels and RA, except for among a small subset of NHSII women with levels measured closest to RA diagnosis.

**Key words:** rheumatoid arthritis, vitamin D, risk factors.

## Introduction

Vitamin D is a hormone with demonstrated immunomodulatory properties influencing a range of immune cells, including T lymphocytes, B lymphocytes and dendritic cells [1, 2]. Low levels of vitamin D have been implicated as an aetiological agent in autoimmune diseases, IBD and multiple sclerosis and may have potential aetiological implications for RA. However, a causal association between

decreased vitamin D levels and increased RA risk has yet to be definitively demonstrated.

Vitamin D's primary mode of action is via binding of its most biologically active form, 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D] to the intracellular vitamin D receptor. The half-life of 1,25(OH)<sub>2</sub>D is only 4 h, hence 25-hydroxyvitamin D [25(OH)D] with a half-life of 2–3 weeks is generally considered the best measure of overall vitamin D status [3].

RA is an inflammatory disease with both inherited and modifiable risk factors. Prior studies examining the association between vitamin D intake and RA have arrived at differing results [4, 5], as have studies utilizing other exposures, such as geographic residence and ultraviolet (UV) exposure, presumably proxies of vitamin D status [6–8]. There has been a single previous study of preclinical RA 25(OH)D levels and the risk of developing RA, which

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Submitted 14 November 2013; revised version accepted 21 May 2014.

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did not observe a difference in the proportion of future RA cases and controls who were vitamin D deficient [9]. However, this study has yet to be replicated.

The objective of our study was to elucidate the relationship between preclinical circulating 25(OH)D and incident RA using two nested case-control studies within the prospective cohort Nurses' Health Study (NHS) and NHS II (NHSII).

## Methods

### Study population

NHS began in 1976 when 121 700 US female registered nurses, 30–55 years of age, were enrolled (birthdates 1921–1946). Of those, 32 826 (27%) participants provided blood samples from May 1989 to September 1990, to be stored for future studies. In 1989 NHSII began with 116 430 female registered nurses, 25–42 years of age at enrolment (birthdates 1947–1964). From 1996 to 1999, 29 613 (25%) participants provided blood samples. Samples have been stored in liquid N<sub>2</sub> freezers ( $\leq -130^{\circ}\text{C}$ ) since collection. Both cohorts are contacted every 2 years by questionnaire to update diet, medications, anthropometrics and incident physician-diagnosed illnesses. Total response rates to the follow-up questionnaires are >90% in each cycle. Deaths in the cohorts are usually reported by next of kin and confirmed by the National Death Index. All participants provided informed consent. All aspects of this study were approved by the Partners Healthcare Institutional Review Board.

### Case identification

Incident cases of RA were identified until the end of follow-up (June 2006 NHS and 2007 NHSII) and confirmed using a two-stage case validation process previously described in detail elsewhere [10]. Participants who self-reported any CTD, including RA, on the biennial follow-up surveys were asked to complete the previously validated CTD Screening Questionnaire [11]. If positive, medical records were independently reviewed by two board-certified rheumatologists, trained in chart abstraction, to confirm the self-reported diagnosis against standardized ACR classification criteria for RA [12].

### Identification of matched controls

Three controls for each confirmed incident RA case were randomly chosen from subjects with stored blood, matched on age ( $\pm 1$  year), menopausal status and postmenopausal hormone use, month and year of blood collection, time of day and fasting status at blood draw. In NHSII, controls were additionally matched to premenopausal cases based on phase in the menstrual cycle.

Participants who were missing dates of birth or death, had confirmed CTD (including RA) before the start of follow-up, had a first symptom of RA prior to blood draw, reported CTD during follow-up but for whom the diagnosis was not confirmed by medical record review and participants with outlier 25(OH)D measurements

falling outside 4 s.d. of the mean observed within controls were excluded.

### 25(OH)D measurement

Circulating 25(OH)D levels were measured in plasma samples in the Heartland laboratory by radioimmunoassay as described by Hollis *et al.* [13]. The plasma samples were randomly sorted within each matched case-control set and the laboratory personnel were blinded to the case/control status of the samples. The time between blood collection and 25(OH)D assay ranged from 10 to 20 years. Prior studies have demonstrated the stability of 25(OH)D in fresh frozen plasma for samples stored for >10 years [14]. Additionally, the mean time from blood draw to RA diagnosis was 7.8 years (s.d. 4.2) for NHS participants and 4.2 years (s.d. 2.0) for NHSII, suggesting that the majority of samples were collected within 10 years. The intra-assay coefficient of variation, determined from blind quality control samples, were good at 9% for both NHS and NHSII.

### Additional covariates

Data on potential confounders of the plasma vitamin D level and RA relationship were collected from biennial questionnaires. Multivariable models included covariates from the questionnaire prior to blood draw, including smoking status, and additional potential confounders based on prior findings in these cohorts [8, 10, 15, 16], parity and breastfeeding, alcohol consumption (0, <5, 5–<9, 9–<15 or  $\geq 15$  g/day), BMI, median income and region of residence in the USA (West, Midwest, Mid-Atlantic, New England and Southeast). A covariate for race was excluded since >98% of the sample self-identified as white.

### Statistical analysis

We used conditional logistic regression, conditioned on matching factors (age, date of blood draw, fasting status, menopausal status, hormone use) to calculate the odds ratio (OR) and 95% CI for incident RA for several measures of exposure, continuous 25(OH)D (each 1 ng/ml increase), sufficient vitamin D (>20 ng/ml) and quartiles of 25(OH)D using unadjusted and multivariable-adjusted conditional logistic regression models. We also tested the hypothesis that the relationship between 25(OH)D and RA risk is not proportional over time using Cox models with a product term including 25(OH)D and follow-up time from blood draw. Based on those results, we repeated the analyses, stratifying cases and controls into subgroups by categories of time between the blood draw and RA diagnosis (3 months–<4 years compared with  $\geq 4$  years) that would optimize our power to detect an association within strata. Fixed effects models were used to meta-analyse estimates of association from the two cohorts given that tests for heterogeneity were non-significant ( $P > 0.1$ ).

## Results

Incident RA was confirmed in 120 NHS and 46 NHSII participants. Mean age at RA diagnosis was 63.8 years (s.d. 8.2) for NHS and 48.5 years (s.d. 4.7) for NHSII participants and 52% were RF positive (Table 1). Mean time from blood draw to RA diagnosis was 7.8 years (s.d. 4.2) for NHS and 4.2 years (s.d. 2.0) for NHSII participants. Meta-analysis of crude and multivariable-adjusted conditional logistic models did not show significant associations between circulating 25(OH)D levels (continuously, dichotomously or in quartiles) and risk of RA (Table 2).

There was evidence that the relationship between 25(OH)D and RA varied over time (product term  $P$ -value < 0.001). We therefore repeated the analyses stratified by time from 25(OH)D measurement to RA diagnosis and observed a 20% decreased risk of developing RA with every 1 ng/ml increase in circulating 25(OH)D [OR 0.80 (95% CI 0.64, 0.99)] among those NHSII women who had blood drawn between 3 months and <4 years prior to the first RA symptom (Table 3). There was no association between 25(OH)D levels and RA in longer time intervals to the first RA symptom, and there was no association between 25(OH)D levels and RA for either time interval in NHS.

## Discussion

There is conflicting evidence for the role of vitamin D in the risk of developing RA. In this study we did not observe an overall prospective association between 25(OH)D levels measured between 3 months and 16 years prior to RA diagnosis and the future development of RA in the combined cohorts or in the NHS cohort. However, there was a significant inverse association between increasing 25(OH)D level and decreased RA risk among a small subset of NHSII women with 25(OH)D levels measured <4 years before the first RA symptom.

Prior studies of the association of vitamin D and RA have used different proxies for vitamin D status. The

source of vitamin D for most people is from dietary and supplement intake, with the majority coming from exposure of the skin to sunlight, specifically ultraviolet B (UVB) rays [3]. Studies of NHS and NHSII cohorts examined the association between geocoded addresses and RA risk and found a generally higher risk of RA among those residing in the Northeast region of the USA [6, 7]. Another study utilizing the same cohorts found that increased UVB exposure was associated with a decreased RA risk among NHS participants [HR 0.79 (95% CI 0.66, 0.94) comparing the highest vs the lowest category of UVB exposure], but was not associated with RA risk among younger women in NHSII [8]. Although UVB exposure is related to vitamin D levels, it is potentially confounded by other associated behaviour, including sun-protective behaviour. Hence UVB exposure likely represents something more than vitamin D levels alone. In fact, sensitivity analyses adding a composite derived index of UVB exposure to our main effects and multivariable models demonstrated no change in the 25(OH)D and RA point estimates and CIs. This may explain the disparate findings between UVB and RA and circulating 25(OH)D and RA within the same cohorts.

Prospective cohort studies of dietary vitamin D intake and RA observed different results. The Iowa Women's Health study found that through 11 years of follow-up, the highest versus the lowest tertile of a single baseline assessment of vitamin D intake was associated with an almost 30% decreased risk of RA [relative risk (RR) 0.67, 95% CI 0.44, 1.00,  $P$ -value for trend = 0.05] [4]. The study of vitamin D intake conducted in NHS and NHSII using 22 years of prospectively collected information with updating of dietary intake variables found no significant association between vitamin D intake and risk of RA [5]. Some of the differences between the two studies' findings may be attributable to demographic differences between the cohorts, such as age, or to modelling the exposure of baseline vitamin D intake compared with updated vitamin D intake, as was done in the previous NHS and NHSII

**TABLE 1** Characteristics of case and control participants in NHS and NHSII

Characteristic	NHS		NHSII	
	Cases ( <i>n</i> = 120)	Controls ( <i>n</i> = 357)	Cases ( <i>n</i> = 46)	Controls ( <i>n</i> = 133)
Age at blood draw, mean (s.d.), years	56.0 (7.1)	56.0 (7.1)	44.4 (4.4)	44.5 (5.3)
Age at diagnosis/censorship, mean (s.d.), years	63.8 (8.2)	63.8 (8.2)	48.5 (4.7)	48.5 (5.1)
Seropositive, %	51	—	57	—
Self-identified race white, %	99	99	98	95
Ever a smoker, %	58	57	41	39
BMI, mean (s.d.), kg/m <sup>2</sup>	25.3 (4.2)	24.6 (4.3)	28.5 (6.3)	25.8 (5.3)
Northeastern region residence, %	23	18	28	19
Alcohol consumption, average (s.d.), g/day	4.5 (5.9)	6.6 (9.4)	2.6 (3.5)	3.0 (5.2)
Physical activity, metabolic equivalent-hours/week (s.d.)	14.3 (14.9)	18.1 (43.5)	13.9 (14.4)	17.5 (21.3)

NHS: Nurses' Health Study; NHSII: Nurses' Health Study II.

**TABLE 2** Estimated odds ratios and 95% CIs of association between 25(OH)D and RA for NHS, NHSII and meta-analysis of both cohorts

	NHS			NHSII			Meta-analysis of NHS and NHSII		
	Cases/ controls	Conditional OR (95% CI) <sup>a</sup>	Multivariable OR (95% CI) <sup>b</sup>	NHSII cases	Minimally adjusted OR (95% CI) <sup>a</sup>	Multivariable OR (95% CI) <sup>b</sup>	Minimally adjusted OR (95% CI) <sup>a</sup>	Multivariable OR (95% CI) <sup>b</sup>	P het <sup>c</sup>
Increase of 1 ng/ ml 25(OH)D	120/357	1.00 (0.98, 1.03)	1.01 (0.99, 1.04)	46/133	0.97 (0.94, 1.01)	0.98 (0.94, 1.03)	0.99 (0.97, 1.01)	1.00 (0.98, 1.03)	0.23
Sufficient 25(OH)D >20 ng/ml	83/241	1.10 (0.70, 1.74)	1.30 (0.78, 2.16)	28/87	0.81 (0.39, 1.68)	0.97 (0.38, 2.50)	1.01 (0.69, 1.49)	1.21 (0.77, 1.90)	0.59
Quartile 1	28/87	1.00	1.00	15/33	1.00	1.00	1.00	1.00	
Quartile 2	34/90	1.23 (0.68, 2.22)	1.26 (0.66, 2.39)	10/33	0.64 (0.24, 1.67)	0.51 (0.15, 1.75)	1.02 (0.62, 1.70)	1.04 (0.59, 1.83)	0.20
Quartile 3	26/90	0.92 (0.49, 1.72)	1.08 (0.55, 2.13)	12/34	0.74 (0.29, 1.89)	0.76 (0.22, 2.59)	0.86 (0.51, 1.45)	0.99 (0.55, 1.80)	0.62
Quartile 4	32/90	1.13 (0.61, 2.10)	1.51 (0.75, 3.05)	9/33	0.60 (0.22, 1.61)	0.66 (0.17, 2.56)	0.94 (0.56, 1.60)	1.26 (0.68, 2.36)	0.29

<sup>a</sup>Conditional logistic regression conducted on cases and controls matched on age ( $\pm$  1 year), menopausal status and postmenopausal hormone use, time of data collection and fasting status at blood draw. NHSII controls additionally matched on timing of blood sample in the menstrual cycle. <sup>b</sup>Multivariable model additionally adjusted for covariates for ever/never smoking, parity and breastfeeding, alcohol consumption, BMI, median income and region of residence in the USA. <sup>c</sup>P het: P-value corresponding to the test for heterogeneity between two cohorts for the multivariable models using the Dersimonian and Laird random effects model. OR: odds ratio; NHS: Nurses' Health Study; NHSII: Nurses' Health Study II; 25(OH)D: 25-hydroxyvitamin D.

**TABLE 3** Estimated odds ratios and 95% CIs for each 1 ng/ml increase in 25(OH)D and RA for NHS, NHSII and meta-analysis of both cohorts

	NHS			NHSII			Meta-analysis of NHS and NHSII		
	Cases/ controls	Minimally ad- justed OR (95% CI) <sup>a</sup>	Multivariable OR (95% CI) <sup>b</sup>	NHSII Cases	Minimally ad- justed OR (95% CI) <sup>a</sup>	Multivariable OR (95% CI) <sup>b</sup>	Minimally ad- justed OR (95% CI) <sup>a</sup>	Multivariable OR (95% CI) <sup>b</sup>	P het <sup>c</sup>
3 months to <4 years	27/80	1.01 (0.96, 1.06)	0.94 (0.85, 1.04)	23/66	0.98 (0.93, 1.03)	0.80 (0.64, 0.99)	0.99 (0.96, 1.03)	0.91 (0.83, 1.00)	0.17
$\geq$ 4 years	93/277	1.00 (0.97, 1.03)	1.02 (0.99, 1.05)	23/67	0.96 (0.91, 1.02)	1.00 (0.92, 1.08)	0.99 (0.97, 1.02)	1.02 (0.99, 1.05)	0.62

<sup>a</sup>Conditional logistic regression conducted on cases and controls matched on age ( $\pm$  1 year), menopausal status and postmenopausal hormone use, time of data collection and fasting status at blood draw. NHSII controls matched on timing of blood sample in the menstrual cycle. <sup>b</sup>Multivariable model additionally adjusted for covariates for ever/never smoking, parity and breastfeeding, alcohol consumption, BMI, median income and region of residence in the USA. <sup>c</sup>P het: P-value corresponding to the test for heterogeneity between two cohorts for the multivariable models using the Dersimonian and Laird random effects model. NHS: Nurses' Health Study; NHSII: Nurses' Health Study II; OR: odds ratio; 25(OH)D: 25-hydroxyvitamin D.

study. These discrepancies may shed light on the relevant exposure window of vitamin D for the development of RA.

A report by Nielen *et al.* [9] included data from 79 RA patients who donated blood a median of 13 times (range 1–51) at a blood bank before the onset of RA symptoms. They compared the proportion of cases and controls who were vitamin D insufficient ( $\leq 20$  ng/ml) at 1, 2 and 5 years prior to RA diagnosis and observed no differences. However, the investigators did not explore linear associations with continuous 25(OH)D or other flexible models of association, such as percentiles, and how these related to RA risk. Similarly, we did not observe a statistically significant association between insufficient vitamin D and RA in NHS or NHSII overall, yet we did observe an inverse linear association between 25(OH)D and RA among the subset of NHSII women closest to RA symptom onset, which may be a chance association since it was not observed among NHS women.

Another study of 1210 healthy individuals at risk for RA based on genetics or family history, with 6.3% being seropositive (RF and/or CCP), found no association between levels of 25(OH)D and seropositivity [17]. This study was conducted among healthy individuals at risk of RA and was cross-sectional in design with antibody and 25(OH)D levels measured simultaneously. It is not known how vitamin D levels and seropositive status jointly affect RA risk in this population.

We acknowledge some limitations of our study. We included women in their 40s and 50s at the start of follow-up. Hence our observations may not be generalizable to younger women or men diagnosed with RA. In our cohorts, only single measurements of 25(OH)D were available for analysis, which precluded any analysis of 25(OH)D levels over time and may not accurately reflect long-term vitamin D status. In our study we excluded all cases with RA symptom onset prior to or within 3 months of blood sampling. However, the timing of the first symptom is based on chart review and could not be confirmed by physical examination. By excluding those individuals with blood drawn after first RA symptom onset, we likely reduced the possibility of confounding by reverse causation compared with excluding those with blood drawn only before RA diagnosis. Lastly, matching within the nested case-control study design that includes a subset of NHS and NHSII women, has the potential to restrict variability in 25(OH)D and hence our power to detect an association with RA. However, matching is a means of controlling for potential confounders of the vitamin D and RA association, such as season and age, which could be associated with both 25(OH)D levels and RA.

A phenomenon of disease phases including a preclinical autoimmune phase has been proposed for autoimmune diseases, including type 1 diabetes and SLE [18]. Similarly, three phases of disease have been hypothesized for RA. Phase 1 is the genetic risk phase, characterized by genetic susceptibility to disease. Phase 2 is the preclinical phase of autoimmunity, when environmental factors presumably interact with genetic susceptibility in the development of RA-related autoantibodies.

Phase 3 is the development of clinically apparent disease [19].

Preclinical RA may result in lower levels of circulating vitamin D due to subclinical inflammatory processes as well as subtle changes in diet and/or decreased sun exposure (changes in physical activity). Although there is good evidence for the immunomodulatory role of vitamin D with impacts on circulating cytokine levels, T cell activation and dendritic cell maturation, differentiation and migration [1, 20, 21], there is also evidence that acute physiological stress and critical illness, via haemodilution, interstitial extravasation or decreased synthesis of binding proteins, augments renal excretion of 25(OH)D, resulting in lower levels of circulating 25(OH)D [22]. Although we observed differences in the relationship between 25(OH)D and RA over time, with an inverse association observed within 4 years of RA diagnosis, it is still difficult to establish with certainty the directionality of the association: is low vitamin D increasing the risk of RA or is preclinical RA inflammation resulting in lower vitamin D levels?

Our study had a number of strengths, including being one of the few studies with prospectively collected samples for 25(OH)D analysis collected prior to RA symptom onset. We were also able to use important potential confounders of the association between vitamin D and RA in our multivariable models, including region of residence, smoking status, alcohol use and BMI, which have demonstrated known associations with both exposure and outcome.

We did not find a significant overall association between circulating 25(OH)D levels and risk of developing RA among women in NHS and NHSII. However, we did detect an increased risk of RA associated with low 25(OH)D among a small subset of women with levels measured closest to RA diagnosis ( $>3$  months– $<4$  years) suggesting that vitamin D levels are lower in the time windows closest to RA diagnosis. Future studies, including prospective intervention trials, would provide important insights into the direction of association between vitamin D and RA.

#### Rheumatology key messages

- Prior studies of vitamin D and risk of RA have come to differing conclusions.
- Prospectively collected 25-hydroxyvitamin D levels before RA diagnosis demonstrated no clear association with risk of RA.

#### Acknowledgements

The authors acknowledge the Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School and the participants in the Nurses' Health Studies. Dr L.T.H.'s work was supported by a Canadian Institute of Health Research Fellowship Award. Dr E.V.A.'s work was

supported by an Arthritis Foundation Dissertation Award. Dr K.H.C.'s work was supported by NIH grant AR059073. Dr E.W.K.'s work was supported by NIH grants AR049880, AR47782 and AR052403.

**Funding:** The Nurses' Health Study and Nurses' Health Study II were supported by NIH grants P01 CA87969, R01 CA49449, UM1 CA176726, R01 CA67262 and R01 CA50385.

**Disclosure statement:** The authors have declared no conflicts of interest.

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