Prediagnostic Serum Vitamin D Levels and the Risk of Crohn's Disease and Ulcerative Colitis in European Populations: A Nested Case-Control Study

Jorrit L. Opstelten, MD, MSc, Simon S.M. Chan, MB BChir, PhD,†,‡ Andrew R. Hart, MB ChB, MD,†,‡ Fiona D.M. van Schaik, MD, PhD,* Peter D. Siersema, MD, PhD,§ Eef G.W.M. Lentjes, PhD,¶ Kay-Tee Khaw, MB BChir,** Robert Luben, BSc,** Timothy J. Key, DPhil,†† Heiner Boeing, PhD,‡‡ Manuela M. Bergmann, PhD,‡‡ Kim Overvad, MD, PhD,§§ Domenico Palli, MD, MPH,¶¶ Giovanna Masala, MD, MPH,¶¶ Antoine Racine, MD, PhD,***,††† Franck Carbonnel, MD, PhD,***,††† Marie-Christine Boutron-Ruault, MD, PhD,*** Anne Tjønneland, MD, PhD, DMSc,‡‡‡ Anja Olsen, PhD,‡‡‡ Vibeke Andersen, MD, PhD,§§§ Rudolf Kaaks, PhD,¶¶¶ Tilman Kühn, PhD,¶¶¶ Rosario Tumino, MD, MSc,**** Antonia Trichopoulou, MD, PhD,†††† Petra H.M. Peeters, MD, PhD,‡‡‡‡,§§§§ W.M. Monique Verschuren, PhD,‡‡‡‡,¶¶¶¶ Ben J.M. Witteman, MD, PhD,*****,††††† and Bas Oldenburg, MD, PhD*

Background: A low vitamin D status has been put forward as a potential risk factor for the development of inflammatory bowel disease (IBD). This study investigated the association between prediagnostic circulating vitamin D concentrations and dietary intakes of vitamin D, and the risk of Crohn's disease (CD) and ulcerative colitis (UC).

Methods: Among 359,728 participants of the European Prospective Investigation into Cancer and Nutrition cohort, individuals who developed CD or UC after enrollment were identified. Each case was matched with2 controls by center, gender, age, date of recruitment, and follow-up time. At cohort entry, blood samples were collected and dietary vitamin D intakes were obtained from validated food frequency questionnaires. Serum 25-hydroxyvitamin D levels were measured using liquid chromatography-tandem mass spectrometry. Conditional logistic regression was performed to determine the odds of CD and UC.

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*Department of Gastroenterology and Hepatology, University Medical Center Utrecht, Utrecht, the Netherlands; †Department of Medicine, Norwich Medical School, University of East Anglia, Norwich, United Kingdom; ‡Department of Gastroenterology, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, United Kingdom; §Department of Gastroenterology and Hepatology, Radboud University Medical Center, Nijmegen, the Netherlands; ¶Department of Clinical Chemistry and Hematology, University Medical Center, Utrecht, the Netherlands; **Strangeways Research Laboratory, Institute of Public Health, University of Cambridge, Cambridge, United Kingdom; ††Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom; ‡‡Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Nuthetal, Germany; §§Section for Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark; ¶¶Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute (ISPO), Florence, Italy; ***Center for Research in Epidemiology and Population Health, French Institute of Health and Medical Research (Inserm), Institut Gustave Roussy, Villejuif, France; †††Department of Gastroenterology, University Hospital of Bicêtre, Assistance Publique – Hôpitaux de Paris, Université Paris-Sud, Le Kremlin Bicêtre, France; ‡‡‡Unit of Diet, Genes and Environment, Danish Cancer Society Research Center, Copenhagen, Denmark; §§§Institute of Regional Research-Center Sønderjylland, University of Southern Denmark, Odense, Denmark; ¶¶¶Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany; ****Cancer Registry and Histopathology Unit, "Civic—M.P. Arezzo" Hospital, Ragusa, Italy; ††††Hellenic Health Foundation, Athens, Greece; ‡‡‡‡Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands; §§§§MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, Imperial College, London, United Kingdom; ¶¶¶¶Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; *****Department of Gastroenterology and Hepatology, Gelderse Vallei Hospital, Ede, the Netherlands; †††††Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands

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Address correspondence to: Department of Gastroenterology and Hepatology, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands. E-mail: boldenbu@umcutrecht.nl

Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; CD, Crohn's disease; CI, confidence interval; EPIC, European Prospective Investigation into Cancer and Nutrition; IBD, inflammatory bowel disease; IQR, interquartile range; LC-MS/MS, liquid chromatography-tandem mass spectrometry; OR, odds ratio; PTH, parathyroid hormone; UC, ulcerative colitis; VDR, vitamin D receptor

Results: Seventy-two participants developed CD and 169 participants developed UC after a median follow-up of 4.7 and 4.1 years, respectively. Compared with the lowest quartile, no associations with the 3 higher quartiles of vitamin D concentrations were observed for CD ($p_{trend} = 0.34$) or UC ($p_{trend} = 0.66$). Similarly, no associations were detected when serum vitamin D levels were analyzed as a continuous variable. Dietary vitamin D intakes were not associated with CD ($p_{trend} = 0.39$) or UC ($p_{trend} = 0.83$).

Conclusions: Vitamin D status was not associated with the development of CD or UC. This does not suggest a major role for vitamin D deficiency in the etiology of IBD, although larger studies are needed to confirm these findings.

Key Words: vitamin D, Crohn's disease, ulcerative colitis, inflammatory bowel disease, etiology

INTRODUCTION

The rising incidence and geographical variability of Crohn's disease (CD) and ulcerative colitis (UC) suggest that environmental factors are involved in the etiology of these inflammatory bowel diseases (IBDs).[1, 2] The role of vitamin D in the pathogenesis of IBD has received increasing interest after the observation that vitamin D receptors (VDRs) are widely expressed throughout the body and that several tissues and cells, including immune cells, synthesize the active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), from the primary circulating form, 25-hydroxyvitamin D (25(OH)D).[3] Apart from well-established effects on calcium homeostasis and bone metabolism, binding of $1,25(OH)_2D$ to the VDR may result in anti-inflammatory effects by modulation of both innate and adaptive immunity.[4]

A low vitamin D status has been proposed as a potential risk factor for the development of IBD based on different lines of evidence.[4] In experimental animal models, vitamin D or VDR deficient mice develop more pronounced intestinal inflammation, whereas supplementation of $1,25(OH)_2D$ suppresses colitis.[4–7] Notably, genetic polymorphisms in the VDR have been implicated in susceptibility to IBD in humans.[8, 9] Experimental studies indicate that vitamin D plays a part in preserving the integrity of the intestinal mucosal barrier.[10, 11] Furthermore, observational studies show that IBD is more common at higher latitudes,[12, 13] where individuals have a lower exposure to ultraviolet B radiation from sunlight, the main determinant of vitamin D status. Vitamin D deficiency is frequently observed in patients with established CD and UC, but this may be a consequence rather than a cause of the disease. Data on prediagnostic vitamin D levels are scant.[14] To date, a single prospective cohort study conducted in women from the United States reported an association between higher vitamin D status and lower risk of incident CD, and to a lesser extent UC.[15] However, a limitation of this study was that these results were based on predicted and not measured circulating 25(OH)D levels, whereas previous studies report that even extensive prediction models may only explain a limited fraction of 25(OH)D variability.[16–18] Prospective studies using accurate measures of vitamin D status including from European populations are not available.

The aim of this study was to investigate the association between prediagnostic circulating vitamin D concentrations measured from serum and dietary intakes of vitamin D, and the risk of CD and UC in a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. We hypothesized that a high vitamin D status decreases the risk of developing IBD.

MATERIALS AND METHODS

Study Population and Data Collection

The EPIC study is a large, multicenter cohort investigation initiated to examine the relationship between lifestyle and environmental factors and the incidence of cancer and other chronic diseases. The design and methods of the EPIC project have been described elsewhere in detail.[19, 20] The cohort used for the present study includes 359,728 men and women aged 20 to 80 years enrolled between 1992 and 2000 from 12 centers in Denmark, France, Germany, Greece, Italy, the Netherlands, and the United Kingdom. Participants were recruited from the general population in all centers, apart from France (female school workers in a nationwide health insurance program), Florence and Utrecht (women in a breast cancer screening program), Ragusa (mainly blood donors), and Oxford (vegetarians, vegans, and other health-conscious individuals). At enrollment, participants completed lifestyle and dietary questionnaires and anthropometric data were collected. In addition, blood samples were taken at cohort entry from most participants before diagnosis. Until further analyses, blood samples were stored at the International Agency for Research on Cancer (Lyon, France) in -196°C liquid nitrogen for all countries except Denmark (-150°C, nitrogen vapor). Participants' habitual diet over the preceding 12 months before recruitment was assessed by validated country-specific food frequency questionnaires. National food composition tables were used to calculate daily intakes of total energy (in kcal), dietary vitamin D (in μg), and other nutrients. Information on supplement use was not available for all participants and was therefore not included.

Follow-up and Identification of Cases and Controls

Participants were followed-up from recruitment until at least May 2004, and in some centers were followed-up to September 2011. Individuals who developed CD or UC were

identified by follow-up questionnaires, population-based disease registries, hospital admission records, pathology databases, health insurance registries, or a combination of these methods.[21] Physicians in each center reviewed the medical records of potential cases to confirm the diagnoses and to obtain information on disease extent according to a standard protocol consistent across centers. Individuals with prevalent CD or UC at enrollment and individuals who developed microscopic or indeterminate colitis were excluded. In this nested case-control study, each case was matched with 2 randomly selected controls by center, gender, age at recruitment (±6 months), and date of recruitment (±3 months). Incidence density sampling was used to establish a similar follow-up time for cases and controls. Only cases (and their controls) with available serum samples were included in the present study.

Laboratory Analysis

The reliability of measuring biomarkers in serum samples collected from the EPIC cohort, including vitamin D, has been investigated previously.[22] Serum samples were analyzed for 25(OH)D and parathyroid hormone (PTH) concentrations. Levels of 25(OH)D were quantified for all cases and controls using liquid chromatography-tandem mass spectrometry (LC-MS/MS). This method was calibrated to the United States National Institute of Standards and Technology. Day-to-day imprecision was 7%, 5%, and 4.3% at 15 nmol/L, 49 nmol/L, and 87 nmol/L, respectively. Within-run imprecision (n = 10) was 3.8%, 3.5%, and 1.7% for these concentrations. PTH levels were measured by an electrochemiluminescence immunoassay on the Modular E411 (intact PTH, Roche Diagnostics GmbH, Mannheim, Germany). The lower limit of detection was 0.60 pmol/L and the intraassay variation was less than 4.5% at 3.5 to 112 pmol/L (reference interval 1 to 7 pmol/L (normal calcium)). All laboratory analyses were performed at the Department of Clinical Chemistry and Hematology at the University Medical Center Utrecht (Utrecht, the Netherlands) in May and June 2016. Laboratory technicians were blinded to the case-control status of participants.

Statistical Analysis

Pearson's correlation coefficient was used to determine the relationship between PTH levels and dietary vitamin D intakes and 25(OH)D levels. Conditional logistic regression models were computed to assess the association between serum 25(OH)D concentrations and dietary vitamin D intakes and the risk of CD and UC by estimating odds ratios (ORs) and 95% confidence intervals (CIs). Levels of 25(OH)D and dietary intakes of vitamin D were introduced in models as quartiles based on the distribution of the matched controls. For circulating 25(OH)D concentrations, analyses also were performed using serum levels as a continuous variable (increment 10 nmol/L) and as a categorical variable based on predefined cutoffs of proposed levels of vitamin D deficiency (less than 50 nmol/L), insufficiency (50 to less than 75 nmol/L), and sufficiency (equal or greater than 75 nmol/L).[3] Restricted cubic spline analyses were performed to evaluate the possibility of a nonlinear association between 25(OH)D levels and the risk of IBD. The likelihood ratio test was used to compare the model with only the linear term to the model with the linear term and the cubic spline terms (5 knots).[23] The p-value of this test was 0.71 for CD and 0.82 for UC, implying no indications of nonlinearity. Therefore, 25(OH)D concentrations were analyzed as a linear term in the analyses with 25(OH)D as a continuous variable. To test for trends across quartiles and categories, trend variables were assigned the median values for these groups. In multivariable models, analyses were adjusted for smoking status (categorized into never smoker, former smoker, and current smoker) since smoking has been consistently associated with CD and UC.[24] Other potential confounders that reportedly were found to be associated[25, 26] or not to be associated[27–29] with IBD, including PTH, linoleic acid, docosahexaenoic acid, body mass index, physical activity, alcohol intake, total dairy products, and dietary calcium intake, were examined but not included in the multivariable models as these factors did not significantly alter the associations. Two analyses were performed to correct for the possible influence of season or month of blood draw on 25(OH)D levels. First, additional adjustment for season of recruitment (winter, spring, summer, or autumn) was performed. Second, 25(OH)D levels were standardized for all participants by deriving residuals from a linear regression model fitted to 25(OH)D concentrations by month of recruitment. The standardized values were then analyzed as a continuous variable in conditional logistic regression models as described above. The results were subsequently compared with those of the nonstandardized 25(OH)D levels.[30] In a sensitivity analysis, the analyses were repeated excluding cases diagnosed less than 18 months after enrollment to minimize the risk of reverse causation. Two-sided p-values below 0.05 were deemed to indicate statistical significance. Statistical analyses were performed using SPSS version 21 (IBM Corp., Armonk, New York, United States) and RStudio version 3.2.2 (RStudio, Inc., Boston, Massachusetts, United States). GraphPad Prism version 6.02 (GraphPad Software, La Jolla, California, United States) was used for drawing figures.

Ethical Considerations

This study was approved by the International Agency for Research on Cancer Ethics Committee and the relevant ethics committees of participating EPIC centers.

RESULTS

In total, 72 participants with incident CD (median age at diagnosis 55.7 years, 77.8% female) and 169 participants with incident UC (median age at diagnosis 57.0 years, 48.5% female) were identified (Table 1). The median time between enrollment and diagnosis was 4.7 years (range 0.1–14.3 years)

TABLE 1: Characteristics of Crohn's Disease and Ulcerative Cases and their Controls

	CD Cases (n = 72)	CD Controls (n = 144)	UC Cases (n = 169)	UC Controls (n = 338)
Female, n (%)	56 (77.8)	112 (77.8)	82 (48.5)	164 (48.5)
Age (years) at recruitment, median (IQR)	49.1 (41.3–60.7)	48.8 (41.6–59.3)	51.6 (46.1–57.9)	51.6 (46.1–57.5)
Age (years) at diagnosis, median (IQR)	55.7 (47.1–63.3)	-	57.0 (51.2–62.3)	-
Distribution of CD, n (%)		-	-	-
L1, ileal	24 (33.3)	-	-	-
L2, colonic	25 (34.7)	-	-	-
L3, ileocolonic	18 (25.0)	-	-	-
L4, upper gastrointestinal disease	3 (4.2)	-	-	-
Unknown	4 (5.6)			
Distribution of UC, n (%)	-	-		-
E1, ulcerative proctitis	-	-	36 (21.3)	-
E2, left-sided colitis	-	-	61 (36.1)	-
E3, extensive colitis	-	-	38 (22.5)	-
unknown			34 (20.1)	
Smoking status, n (%)				
never smoker	23 (31.9)	62 (43.1)	39 (23.1)	115 (34.0)
former smoker	22 (30.6)	34 (23.6)	69 (40.8)	96 (28.4)
current smoker	25 (34.7)	48 (33.3)	55 (32.5)	121 (35.8)
unknown	2 (2.8)	0 (0)	6 (3.6)	6 (1.8)
PTH level (pmol/L), median (IQR)	2.7 (2.1–3.7)	2.7 (2.1–3.5)	2.9 (2.3–3.6)	3.0 (2.3–3.9)
unknown, n (%)	5 (6.9)	7 (4.9)	9 (5.3)	33 (9.8)
25(OH)D level (nmol/L), median (IQR)	59.1 (40.3–73.1)	60.4 (44.1–77.6)	54.2 (39.2–72.9)	54.9 (37.5–70.1)
Dietary vitamin D intake (μg/day), median (IQR)	3.46 (2.00–4.45)	3.03 (2.21–4.10)	3.10 (2.05–4.13)	3.09 (1.94–4.50)
unknown, n (%)	0 (0)	0 (0)	1 (0.6)	3 (0.9)

25(OH)D: 25-hydroxyvitamin D; CD: Crohn's disease; IQR: interquartile range; PTH: parathyroid hormone; UC: ulcerative colitis

and 4.1 years (range 0.1–15.7 years) for CD and UC, respectively. Twenty-five CD cases (34.7%) suffered from colonic disease and 61 UC cases (36.1%) had left-sided colitis. The median 25(OH)D serum level was 59.1 nmol/L (interquartile range [IQR] 40.3–73.1) for CD cases and 60.1 nmol/L (IQR 44.1–77.6) for their controls. The corresponding values for UC cases and their controls were 54.2 nmol/L (IQR 39.2–72.9) and 54.9 nmol/L (IQR 37.5–70.1), respectively. The distribution of 25(OH)D concentrations of CD and UC cases and their controls is shown in Figure 1. According to predefined definitions of vitamin D status, 36.1% of CD cases and 34.7% of their controls had 25(OH)D levels consistent with vitamin D deficiency (less than 50 nmol/L), whereas 44.4% of UC cases and 43.2%. of their controls were vitamin D deficient. PTH concentrations correlated significantly with 25(OH)D concentrations, but the strength of the correlations was weak ($P < 0.01$ and r = -0.20 in CD cases and their controls, $P < 0.01$ and r = -0.22 in UC cases and their controls). The median daily dietary intakes of vitamin D were 3.46 μg (IQR 2.00–4.45) for CD cases, 3.03 μg (IQR 2.21–4.10) for CD controls, 3.10 μg (IQR 2.05–4.13) for UC cases, and 3.09 μg (IQR 1.94–4.50) for UC controls. Both the distribution of 25(OH)D levels and dietary vitamin D intakes did not significantly differ between cases and controls. There were no significant correlations between dietary intakes of vitamin D and 25(OH)D concentrations ($P = 0.87$ and r = -0.01 in CD cases and their controls, $P = 0.82$ and r = 0.01 in UC cases and their controls).

In the multivariable analyses, compared with the lowest quartile, there were no significant associations with the 3 higher quartiles of 25(OH)D levels for CD ($p_{trend} = 0.34$) or UC ($p_{trend} = 0.66$) (Table 2). The analysis based on predefined categories of vitamin D deficiency, insufficiency, and sufficiency also showed no significant associations for CD ($p_{trend} = 0.58$) or UC ($p_{trend} = 0.94$) (Table 2). Similarly, when analyzing 25(OH)D levels as a continuous variable, serum concentrations were not associated with the development of IBD (OR of 0.99 [95% CI 0.88–1.11] per 10 nmol/L for CD and 1.01 [95% CI 0.93–1.11] per 10 nmol/L for UC). Additional adjustment for season of recruitment did not considerably change the magnitudes or directions of the effect sizes. Analyses based on 25(OH)D levels standardized by month of recruitment showed similar results compared with those of the nonstandardized values.

Overall, dietary intakes of vitamin D were not associated with the risk of CD or UC and no significant trends across quartiles were observed, although individuals with a dietary vitamin D intake in the second quartile were found to have a lower risk of CD and a higher risk of UC as compared to individuals in the first and lowest quartile with an OR of 0.34 (95% CI 0.13–0.92) and 1.81 (95% CI 1.02–3.23) in the multivariable analysis of CD and UC, respectively (Table 3).

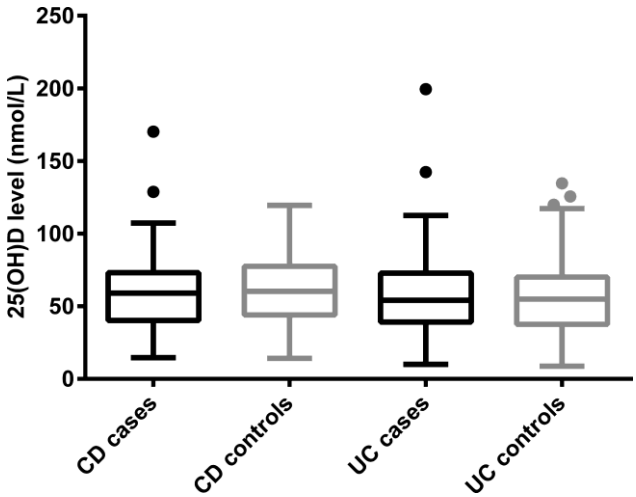

FIGURE 1. Distribution of serum 25(OH)D concentrations of IBD cases and their controls.

In addition, 25(OH)D concentrations or dietary vitamin D intakes were not found to be associated with disease site in either CD or UC (data not shown). When excluding cases diagnosed less than 18 months after cohort entry to minimize the risk of reverse causation, a slightly stronger inverse gradient across quartiles of 25(OH)D levels was observed in the analysis of CD (OR for highest versus lowest quartile 0.59, 95% CI 0.23–1.51, $p_{trend} = 0.24$), but no significant associations between 25(OH)D concentrations or dietary vitamin D intakes and CD or UC were observed (Supplementary Table 1).

DISCUSSION

In this case-control study nested within a large European prospective cohort, prediagnostic 25(OH)D concentrations measured from serum were not associated with the development of CD or UC. The lack of statistically significant associations

TABLE 2: Odds of CD and UC According to 25(OH)D Levels

	Cases, n (%)	Controls, n (%)	Unadjusted OR (95% CI)	Adjusted OR_a (95% CI)
CD				
Quartile of 25(OH)D				
1 (≤44.0 nmol/L)	19 (26.4)	36 (25.0)	1.00	1.00
2 (44.1–60.3 nmol/L)	21 (29.2)	36 (25.0)	1.08 (0.52–2.25)	0.94 (0.43–2.04)
3 (60.4–77.5 nmol/L)	18 (25.0)	36 (25.0)	0.94 (0.41–2.16)	0.79 (0.33–1.89)
4 (≥77.6 nmol/L)	14 (19.4)	36 (25.0)	0.74 (0.32–1.70)	0.69 (0.29–1.60)
			$p_{trend} = 0.41$	$p_{trend} = 0.34$
Predefined category of 25(OH)D				
Deficiency (<50.0 nmol/L)	26 (36.1)	50 (34.7)	1.00	1.00
Insufficiency (50.0–74.9 nmol/L)	30 (41.7)	55 (38.2)	1.06 (0.52–2.18)	1.07 (0.51–2.24)
Sufficiency (≥75.0 nmol/L)	16 (22.2)	39 (27.1)	0.79 (0.36–1.71)	0.80 (0.36–1.77)
			$p_{trend} = 0.54$	$p_{trend} = 0.58$
UC				
Quartile of 25(OH)D				
1 (≤37.5 nmol/L)	37 (21.9)	85 (25.1)	1.00	1.00
2 (37.6–54.9 nmol/L)	48 (28.4)	85 (25.1)	1.28 (0.75–2.16)	1.28 (0.74–2.20)
3 (55.0–70.1 nmol/L)	34 (20.1)	84 (24.9)	0.96 (0.53–1.73)	1.03 (0.56–1.89)
4 (≥70.2 nmol/L)	50 (29.6)	84 (24.9)	1.37 (0.78–2.40)	1.22 (0.67–2.20)
			$p_{trend} = 0.38$	$p_{trend} = 0.66$
Predefined category of 25(OH)D				
Deficiency (<50.0 nmol/L)	75 (44.4)	146 (43.2)	1.00	1.00
Insufficiency (50.0–74.9 nmol/L)	54 (32.0)	130 (38.5)	0.83 (0.53–1.28)	0.83 (0.52–1.31)
Sufficiency (≥75.0 nmol/L)	40 (23.7)	62 (18.3)	1.26 (0.74–2.12)	1.09 (0.62–1.90)
			$p_{trend} = 0.57$	$p_{trend} = 0.94$

25(OH)D: 25-hydroxyvitamin D; CI: confidence interval; OR: odds ratio
[a]adjusted for smoking status

TABLE 3: Odds of CD and UC According to Dietary Vitamin D Intake

	Cases, n (%)	Controls, n (%)	Unadjusted OR (95% CI)	Adjusted OR_a (95% CI)
CD				
Quartile of dietary vitamin D				
1 (≤2.21 μg/day)	24 (33.3)	36 (25.0)	1.00	1.00
2 (2.22–3.03 μg/day)	7 (9.7)	36 (25.0)	0.32 (0.12–0.82)	0.34 (0.13–0.92)
3 (3.04–4.10 μg/day)	18 (25.0)	36 (25.0)	0.78 (0.36–1.67)	0.94 (0.43–2.08)
4 (≥4.11 μg/day)	23 (31.9)	36 (25.0)	1.04 (0.41–2.66) $p_{trend} = 0.48$	1.08 (0.41–2.85) $p_{trend} = 0.39$
UC				
Quartile of dietary vitamin D				
1 (≤1.94 μg/day)	30 (17.9)	83 (24.8)	1.00	1.00
2 (1.95–3.09 μg/day)	54 (32.1)	84 (25.1)	1.82 (1.04–3.16)	1.81 (1.02–3.23)
3 (3.10–4.50 μg/day)	50 (29.8)	84 (25.1)	1.76 (0.95–3.26)	1.83 (0.97–3.45)
4 (≥4.51 μg/day)	34 (20.2)	84 (25.1)	1.17 (0.60–2.29) $p_{trend} = 0.85$	1.15 (0.58–2.29) $p_{trend} = 0.83$

CI: confidence interval; OR: odds ratio
[a]adjusted for smoking status

was consistent when circulating 25(OH)D levels were analyzed either as categorical or continuous variables. Similarly, dietary vitamin D intakes were not associated with the risk of incident CD or UC. Although based on relatively small numbers of cases, these findings are not supportive of a major role for vitamin D status in the etiology of IBD.

Different studies have suggested vitamin D deficiency as a risk factor for the development of IBD.[4] First, epidemiological data indicate that vitamin D deficiency is widespread, especially throughout western populations, and appears to rise in parallel with the incidence of CD and UC.[1, 31, 32] Second, ecological studies have demonstrated that the incidence of IBD is greater at higher latitudes,[12, 13] which could be explained by reduced exposure to ultraviolet B radiation from sunshine and consequently lower vitamin D status. However, latitude may not necessarily directly correlate with sunlight exposure or vitamin D levels[33, 34] and may also reflect other factors, such as affluence or diet.[12, 35, 36] Third, vitamin D intake or status has been linked to an increased risk of other immunologically mediated diseases that may share epidemiological and pathogenic aspects with IBD, such as multiple sclerosis,[37] rheumatoid arthritis,[38] and type 1 diabetes.[39] Fourth, various genetic epidemiological studies have attributed susceptibility to IBD to polymorphisms in the VDR region, although data are not consistent.[8, 9, 40, 41]

A potential link between vitamin D deficiency and the pathogenesis of IBD also is supported by laboratory studies. Most immune cells have a VDR, are able to produce the active form of vitamin D, $1,25(OH)_2D$, from the main circulating form, 25(OH)D, and are found to respond to this local synthesis by exerting anti-inflammatory effects.[4] For example, activation of Toll-like receptors has shown to trigger production of antimicrobial peptides mediated through vitamin D.[42] Other studies have reported that vitamin D modulates proliferation of T-helper 1 cells and T-helper 2 cells.[43] Vitamin D may also inhibit differentiation and maturation of dendritic cells,[44] whereas induction of immunosuppressive regulatory T cells is promoted.[45] In different experimental mouse models, vitamin D or VDR deficiency has shown to result in more severe colitis, which may be prevented or ameliorated by administration of $1,25(OH)_2D$.[4–7] Caution in interpreting these results, however, is warranted since responses in mouse models do not always apply to human inflammatory diseases.[46, 47]

In order to establish a causal relationship, the exposure of interest must precede the outcome. Vitamin D deficiency is common in both patients with CD and UC.[14] However, there are no prospective studies examining the association between vitamin D status before diagnosis and the subsequent development of CD and UC, apart from a single large, prospective cohort study from the United States.[15] In this investigation, higher predicted 25(OH)D levels were significantly associated with a reduced risk of incident CD. A weaker, nonsignificant inverse association between levels of 25(OH)D and risk of incident UC was reported. There was a significant inverse association between dietary and supplemental vitamin D intake and UC and a nonsignificant inverse association with CD.[15] Participants were exclusively female nurses, which potentially limits the generalizability of the findings. However, the main weakness of this study was that the vitamin D status was based on predicted and not measured 25(OH)D levels. A validated regression model was developed to predict the plasma 25(OH)D level of subjects by using a set of lifestyle factors. Although using a predicted 25(OH)D level as a marker for vitamin D

status may be useful in studies with large populations, it has previously been demonstrated that even a comprehensive set of determinants, including ultraviolet B radiation exposure, may account for only around 20% to 30% of circulating 25(OH) D variability.[16–18] Thus, the reliability of predicted circulating 25(OH)D concentrations is modest at best. Residual confounding may be an explanation for the disparity between previous data and the present study. For example, the slight contribution of vitamin D intake to an individual's serum vitamin status may cast doubt on the causality of the reported inverse association of dietary and supplemental vitamin D intake with IBD.[15] This is underscored by the observation that dietary vitamin D intake did not correlate with 25(OH)D concentrations in the present study. Intake of vitamin D or a set of lifestyle determinants as a predictor for 25(OH)D may thus act as a proxy for other factors possibly associated with IBD, such as health-conscious behavior, hygiene, social status, or other yet undefined influences. It may also be that vitamin D deficiency does not increase the risk of developing CD or UC, but rather influences the course of disease once IBD has established. Recent studies have indicated that low 25(OH)D levels are associated with worse clinical outcomes in CD and UC,[48, 49] although a low vitamin D status also could be a mere consequence of (active) disease. Finally, despite the relatively large cohort size of the present study, the numbers of cases may have been insufficient to detect modest associations. Especially for CD, there appeared to be a trend of an inverse association of 25(OH)D levels with CD risk. Nonetheless, if a small effect on risk of IBD associated with vitamin D status exists, the clinical relevance remains uncertain given the low absolute risk of CD and UC.

The present study is the first prospective investigation to explore the putative role of vitamin D in the etiology of IBD using actual concentrations of vitamin D in blood. The 25(OH) D levels observed in this study are comparable to those found in previous epidemiological studies from general European populations,[31] whereas relatively higher levels have been reported in North America.[15, 50] The main strengths of this study were its prospective nature, which reduces the likelihood of selection and reverse causality biases, and the direct measurement of serum 25(OH)D concentrations rather than a proxy marker. Data on the use of vitamin D supplements were not available for all participants and could therefore not be included in this study. However, measurement of 25(OH)D overcomes the lack of information on supplement use, since serum 25(OH)D incorporates vitamin D derived from endogenous production and from dietary intake or supplement use and can therefore be considered the best assessment of an individual's vitamin D status.[30, 51] Concentrations of vitamin D in blood are thus the relevant exposure measure when investigating the potential role of vitamin D in IBD or other diseases. Local physicians confirmed all cases, and individuals who developed microscopic or indeterminate colitis were excluded. The odds of CD and UC were assessed separately and results were either matched or adjusted for potential confounders, such as date of enrollment and smoking. Lastly, data were collected from men and women from geographically diverse populations in Europe, increasing the generalizability of the results, together with including standard ranges for vitamin D and all disease sites of IBD.

There were several limitations to this study. First, the assessment of 25(OH)D and dietary vitamin D intake included just 1 measurement. However, previous studies have shown that a single assessment of serum 25(OH)D has reasonable reliability with regard to long-term variation. Within a previous EPIC study, for example, it was found that serum 25(OH)D levels collected from 2 Dutch centers at 2 time points, several years apart, did not significantly change over time with a percentage change in mean values of 8.2% for men and 12.4% for women.[22] Furthermore, data indicate that general food patterns of adults remain relatively stable over time.[52, 53] Second, as referred to earlier, the relatively small numbers of cases were an important drawback of this study, although this only appeared to hold for cases with CD. Although these results do not exclude the possibility of a small effect on IBD risk associated with vitamin D status, any weak association brings into question the clinical implications. Third, most participants developed CD or UC at around 55 years, whereas these disorders are commonly diagnosed in the second or third decade of life. The results may therefore not apply to the whole IBD population, although the aspects of generalizability were that both men and women were studied and standard determinant of assessments of vitamin D status across different age groups was used. Moreover, the proportion of older patients with IBD is increasing,[54] and environmental factors appear to play a stronger role than genetics in the etiology of IBD in these individuals compared with younger patients with IBD. Fourth, results were corrected for season and month of recruitment, but temporal variation of 25(OH)D levels may have influenced the outcomes. Finally, residual confounding could not be fully excluded in this observational study.

In conclusion, prediagnostic 25(OH)D concentrations measured from serum and dietary intakes of vitamin D were not associated with the development of CD or UC in this observational study in European populations. These findings do not suggest a major role for vitamin D status in the etiology of IBD. Larger studies are needed to confirm these results.

SUPPLEMENTARY DATA

Supplementary data is available at *Inflammatory Bowel Diseases* online.

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