

Prediagnostic serum vitamin D concentrations and the risk of Crohn's disease and ulcerative colitis: a pan-European, nested case-control study.

Short title: Serum vitamin D levels and risk of inflammatory bowel disease.

Jorrit L. Opstelten¹, Simon S.M. Chan^{2,3}, Andrew R. Hart^{2,3}, Fiona D.M. van Schaik¹, Peter D. Siersema⁴, Eef G.W.M. Lentjes⁵, Kay-Tee Khaw⁶, Robert Luben⁶, Timothy J. Key⁷, Heiner Boeing⁸, Manuela M. Bergmann⁸, Kim Overvad⁹, Domenico Palli¹⁰, Giovanna Masala¹⁰, Antoine Racine^{11,12}, Franck Carbonnel^{11,12}, Marie-Christine Boutron-Ruault¹¹, Anne Tjønneland¹³, Anja Olsen¹³, Vibeke Andersen¹⁴, Rudolf Kaaks¹⁵, Verena A. Katzke¹⁵, Rosario Tumino¹⁶, Antonia Trichopoulou¹⁷, Ben J.M. Witteman^{18,19}, Bas Oldenburg¹

Affiliations:

1. Department of Gastroenterology and Hepatology, University Medical Centre Utrecht, Utrecht, the Netherlands
2. Department of Medicine, Norwich Medical School, University of East Anglia, Norwich, United Kingdom
3. Department of Gastroenterology, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, United Kingdom
4. Department of Gastroenterology and Hepatology, Radboud University Medical Centre, Nijmegen, the Netherlands

5. Department of Clinical Chemistry and Haematology, University Medical Centre, Utrecht, the Netherlands
6. Strangeways Research Laboratory, Institute of Public Health, University of Cambridge, Cambridge, United Kingdom
7. Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, United Kingdom
8. Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE), Nuthetal, Germany
9. Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus, Denmark
10. Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute (ISPO), Florence, Italy
11. French Institute of Health and Medical Research (INSERM), Centre for Research in Epidemiology and Population Health, Institut Gustave Roussy, Villejuif, France
12. Department of Gastroenterology, University Hospital of Bicêtre, Assistance Publique Hôpitaux de Paris, Université Paris-Sud, Le Kremlin Bicêtre, France
13. Danish Cancer Society Research Centre, Copenhagen, Denmark
14. Institute of Regional Research, Center Sønderjylland, University of Southern Denmark, Odense, Denmark
15. Division of Cancer Epidemiology, German Cancer Research Centre (DKFZ), Heidelberg, Germany
16. Cancer Registry and Histopathology Unit, "Civic—M.P. Arezzo" Hospital, Ragusa, Italy

17. Department of Hygiene and Epidemiology, WHO Collaborating Centre for Food and Nutrition Policies, University of Athens, Athens, Greece
18. Department of Gastroenterology and Hepatology, Gelderse Vallei Hospital, Ede, the Netherlands
19. Division of Human Nutrition, Wageningen University, Wageningen, the Netherlands

Corresponding author:

B. Oldenburg, MD, PhD

Department of Gastroenterology and Hepatology

University Medical Centre Utrecht

P.O. Box 85500, 3508 GA Utrecht

The Netherlands

E-mail: boldenbu@umcutrecht.nl

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Summary box

What is already known about this subject?

- Observational and experimental studies have suggested that vitamin D deficiency contributes to the development of inflammatory bowel disease (IBD).
- Prospective data on directly measured circulating vitamin D concentrations and the risk of incident Crohn's disease (CD) and ulcerative colitis (UC) are lacking and a causal role for vitamin D status in these intestinal disorders has yet to be established.

What are the new findings?

- Prediagnostic 25-hydroxyvitamin D levels measured from serum are not associated with the development of CD or UC in this observational study in European populations.
- Dietary intakes of vitamin D are not associated with the odds of incident CD or UC in this prospective investigation.

How might it impact on clinical practice in the foreseeable future?

- The results from this study cast doubt on the role of vitamin D status in the aetiology of IBD and its possible relevance in clinical practice.

Abstract (up to 250 words!)

Objective: A causal link between a low vitamin D status and the development of inflammatory bowel disease (IBD) has previously been suggested. This study aimed to investigate 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).

Design: Within the European Prospective Investigation into Cancer and Nutrition cohort, individuals who developed CD or UC after enrolment were identified. Each case was matched with two controls by centre, 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.

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 three higher quartiles of vitamin D concentrations were observed for CD ($p_{\text{trend}} = 0.34$) or UC ($p_{\text{trend}} = 0.66$). Similarly, no associations were detected when serum vitamin D levels were analysed as a continuous variable. Dietary vitamin D intakes were not associated with CD ($p_{\text{trend}} = 0.39$) or UC ($p_{\text{trend}} = 0.93$).

Conclusion: Vitamin D status was not associated with the development of incident CD or UC. These findings cast doubt on the role of vitamin D deficiency in the aetiology of IBD.

Keywords: vitamin D, 25-hydroxyvitamin D, inflammatory bowel disease, Crohn's disease, ulcerative colitis, aetiology

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

Introduction

The rising incidence and geographical variability of Crohn's disease (CD) and ulcerative colitis (UC) suggest that environmental factors are involved in the aetiology 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, synthesise the active form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$), from the primary circulating form, 25-hydroxyvitamin D ($25(\text{OH})\text{D}$) (3). Apart from well-established effects on calcium homeostasis and bone metabolism, binding of $1,25(\text{OH})_2\text{D}$ to the VDR may result in anti-inflammatory effects by modulation of both innate and adaptive immunity (4).

A causal link between a low vitamin D status and the development of IBD has been suggested by different lines of evidence. In experimental animal models, vitamin D or VDR deficient mice develop accelerated intestinal inflammation, whereas supplementation of $1,25(\text{OH})_2\text{D}$ suppresses colitis (5-11). Notably, genetic polymorphisms in the VDR have been implicated in susceptibility to IBD in humans (12-14). Experimental studies indicate that vitamin D plays a part in preserving the integrity of the intestinal mucosal barrier (15,16). Furthermore, observational studies show that IBD is commoner at higher latitudes (17-21), where individuals may have low exposure to ultraviolet B radiation from sunlight, the main determinant of vitamin D status.

Although vitamin D deficiency is frequently observed in patients with established CD and UC, this may be a consequence rather than a cause of the disease. Data on prediagnostic

vitamin D levels are scant (22). To date, a single prospective cohort study, conducted in women from the United States, has reported an association between higher vitamin D status and lower risk of incident CD, and to a lesser extent, UC (23). However, a limitation of the methodology 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 (24-26). Prospective studies using accurate measures of vitamin D status including from European populations do not exist.

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. The findings would help clarify if vitamin D status is involved in the aetiology of IBD.

Methods

Study population and data collection

The EPIC study is a large, multicentre 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 (27-29). The cohort used for the present study includes 359,728 men and women aged 20 to 80 years enrolled between 1992 and 2000 from twelve centres in Denmark, France, Germany, Greece, Italy, the Netherlands and the United Kingdom. Participants were recruited from the general population in all centres, 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 enrolment, 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 vapour). Participants' habitual diet over the preceding twelve months prior to 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 recorded.

Follow-up and identification of cases and controls

Participants were followed up from recruitment until at least May 2004 and in some centres up to September 2011. Individuals who developed a new diagnosis of 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. Physicians in each centre reviewed the medical records of potential cases to confirm the diagnoses and to obtain information on the investigations used for diagnosis and to assess disease extent.

Individuals with prevalent CD or UC at enrolment and individuals who developed microscopic or indeterminate colitis were excluded. In this nested case-control study, each case was matched with two randomly selected controls by centre, 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 analyses

The reliability of measuring biomarkers in serum samples collected from the EPIC cohort, including vitamin D, has been investigated previously (30). Serum samples were analysed 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 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 intra-assay variation was less than 4.5% at 3.5 to 112 pmol/L (reference interval 1 to 7 pmol/L (normal calcium)). Laboratory analyses were performed at the Department of Clinical Chemistry and Haematology at the University Medical Centre Utrecht (Utrecht, the Netherlands). 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 were also performed using serum levels as a continuous variable (increment 10 nmol/L) and as a categorical variable based on predefined cut-offs 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 incidence 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 (five knots). The p-value of this test was 0.71 for CD and 0.82 for UC, indicating that there were no signs of nonlinearity. Therefore, 25(OH)D concentrations were analysed 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 (31). Other potential confounders which reportedly were found to be associated (32,33) or not to be associated (34,35) 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 approaches were used 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, autumn) was performed. Second, 25(OH)D levels were standardised for all subjects by deriving residuals from a linear regression model fitted to 25(OH)D concentrations by month of recruitment. The standardised values were then analysed as a continuous variable in conditional logistic regression models as described above. The results were subsequently compared with those of the nonstandardised 25(OH)D levels (36). In a sensitivity analysis, the analyses were repeated excluding cases diagnosed less than 18 months after enrolment to minimise 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).

Ethical considerations

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

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 enrolment and diagnosis was 4.7 years (range 0.1-14.3 years) 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 for CD cases and 60.1 nmol/L for their controls. The corresponding values for UC cases and their controls were 54.2 nmol/L and 54.9 nmol/L, 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 ($r = -0.20$ in CD cases and their controls, $r = -0.22$ in UC cases and their controls). The median daily dietary intakes of vitamin D were 3.46 μg for CD cases, 3.03 μg for CD controls, 3.10 μg for UC cases and 3.09 μg 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 ($r = -0.01$ in CD cases and their controls, $r = 0.01$ in UC cases and their controls).

In the multivariable analyses, compared with the lowest quartile, there were no significant associations with the three higher quartiles, or trends across quartiles of 25(OH)D

levels for CD ($p_{\text{trend}} = 0.34$) or UC ($p_{\text{trend}} = 0.66$) (Table 2). The analysis based on predefined categories of vitamin D deficiency, insufficiency and sufficiency also showed no significant associations for CD ($p_{\text{trend}} = 0.58$) or UC ($p_{\text{trend}} = 0.94$) (Table 2). Similarly, when analysing 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 0.93-1.1] 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 standardised by month of recruitment showed similar results compared with those of the nonstandardised 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 as compared to individuals in the first and lowest quartile with an OR of 0.34 (95% CI 0.13-0.92) in the multivariable analysis of CD (Table 3).

There were also no significant associations for 25(OH)D concentrations or dietary vitamin D according to disease site for both CD and UC (data not shown). When excluding cases diagnosed less than 18 months after cohort entry in order to minimise 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, $p_{\text{trend}} = 0.24$), 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 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 was consistent when circulating 25(OH)D levels were analysed either as categorical or continuous variables. Similarly, dietary vitamin D intakes were not associated with the risk of incident CD or UC. These findings are therefore not supportive of a major role for vitamin D status in the aetiology of IBD.

A large number of studies suggest that vitamin D may be implicated in the pathogenesis 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 (1,37-39). Second, ecological studies have demonstrated that the incidence of IBD is greater at higher latitudes (17-21), 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 (40,41), and may also reflect other factors, such as affluence or diet (19,42,43). Third, vitamin D status or intake has been linked with an increased risk of other immunologically mediated diseases that may share epidemiological and pathogenic aspects with IBD, such as multiple sclerosis (44), rheumatoid arthritis (45), and type 1 diabetes (46). Fourth, various genetic epidemiological studies have associated polymorphisms in the VDR region to susceptibility to IBD, although data have been conflicting (12-14,47,48).

A link between vitamin D deficiency and the pathogenesis of IBD is also supported by observational and experimental laboratory studies. Most immune cells have a VDR, are able to produce the active form of vitamin D, $1,25(\text{OH})_2\text{D}$, from the main circulating form, $25(\text{OH})\text{D}$, and are found to respond to this local synthesis by exerting anti-inflammatory effects on innate and adaptive immunity (4). For example, activation of Toll-like receptors has shown to trigger an antimicrobial response mediated through vitamin D by production of antimicrobial peptides (49,50). Other studies have reported that vitamin D reduces proliferation of pro-inflammatory T-helper 1 cells and promotes an increase in anti-inflammatory T-helper 2 cells (51). Vitamin D may also inhibit differentiation and maturation of dendritic cells (52), whereas induction of immunosuppressive regulatory T cells is promoted (53). 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 $1,25(\text{OH})_2\text{D}$ (5-11). Similarly, vitamin D and the VDR appear to be important factors in maintaining the integrity of the intestinal mucosal barrier (15,16). Caution in interpreting these results is, however, warranted since responses in mouse models do not always apply to human inflammatory diseases (54,55).

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 (22), including in those recently diagnosed with IBD (56), suggesting that inadequate concentrations may not be a mere consequence of disease. 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 (23). In this investigation, higher predicted $25(\text{OH})\text{D}$ 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 (23). Participants were exclusively female nurses, which potentially limits the generalisability 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 predictors. 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 only account for between approximately 20 and 30% of circulating 25(OH)D variability (24-26). Thus, the ability to predict circulating 25(OH)D concentrations is modest. Residual confounding may be an explanation for the associations found in the aforementioned study (23). A set of lifestyle predictors may be a proxy for other variables possibly associated with IBD, such as health-conscious behaviour, hygiene, social status or other yet undefined influences. The relatively small impact of a high dietary and/or supplemental intake of vitamin D on serum 25(OH)D levels does therefore cast doubt on the causality of the reported inverse association of vitamin D with IBD. This is underscored by our observation that dietary intake of vitamin D did not correlate with 25(OH)D concentrations.

To the best of our knowledge, the present study is the first prospective investigation exploring the association between measured circulating 25(OH)D concentrations and the risk of incident CD and UC. No significant association between vitamin D status and the development

of IBD was observed. A potential explanation for this discrepancy with other data might be misclassification of vitamin D status (actual lack of vitamin D deficiency) in previous studies or that vitamin D deficiency is a proxy for other factors, such as poor health, lifestyle, or country-dependent healthcare characteristics.

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. 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 we were able to either match or adjust for potential confounders, such as date of enrolment and smoking. Finally, data were collected from men and women from geographically diverse populations in Europe, increasing the generalisability of our findings, 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 one measurement. However, previous studies have shown that a single assessment of serum 25(OH)D has reasonable reliability with regard to long-term variation and general food patterns of adults remain relatively stable over time (57-59). Second, most participants developed CD or UC at an age of around 55 years, indicating late-onset IBD, 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 generalisability were that both men and women were studied and standard determinant of assessments of vitamin D status across different age groups were used. Third, despite the

relatively large size of the population in this study, the numbers of cases may have been insufficient to detect subtle associations. Especially for CD, a small inverse biological gradient for 25(OH)D levels might exist. Nonetheless, considering the low absolute risk of CD and UC, any modest associations bring into question the clinical implications in daily practice. Fourth, we corrected for season of recruitment, but temporal variation of 25(OH)D levels may have influenced our results. 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 cast doubt on the role of vitamin D status in the aetiology of IBD and its possible relevance in clinical practice.

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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 Crohn's disease, 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 ulcerative colitis, n (%)				
E1, ulcerative proctitis	-	-	36 (21.3)	-
E2, left-sided colitis	-	-	61 (36.1)	-
E3, extensive colitis	-	-	38 (22.5)	-
unknown	-	-	34 (20.1)	-

Table 1. (continued)

	CD cases (n=72)	CD controls (n=144)	UC cases (n=169)	UC controls (n=338)
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

Table 2. Odds of Crohn's disease and ulcerative colitis according to 25(OH)D levels

	Cases, n (%)	Controls, n (%)	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Crohn's disease				
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)
			$p_{\text{trend}} = 0.41$	$p_{\text{trend}} = 0.34$
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)
			$p_{\text{trend}} = 0.54$	$p_{\text{trend}} = 0.58$
Ulcerative colitis				
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)
			$p_{\text{trend}} = 0.38$	$p_{\text{trend}} = 0.66$
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)
			$p_{\text{trend}} = 0.57$	$p_{\text{trend}} = 0.94$

25(OH)D: 25-hydroxyvitamin D; CI: confidence interval; OR: odds ratio

* adjusted for smoking status

Table 3. Odds of Crohn's disease and ulcerative colitis according to dietary vitamin D intake

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

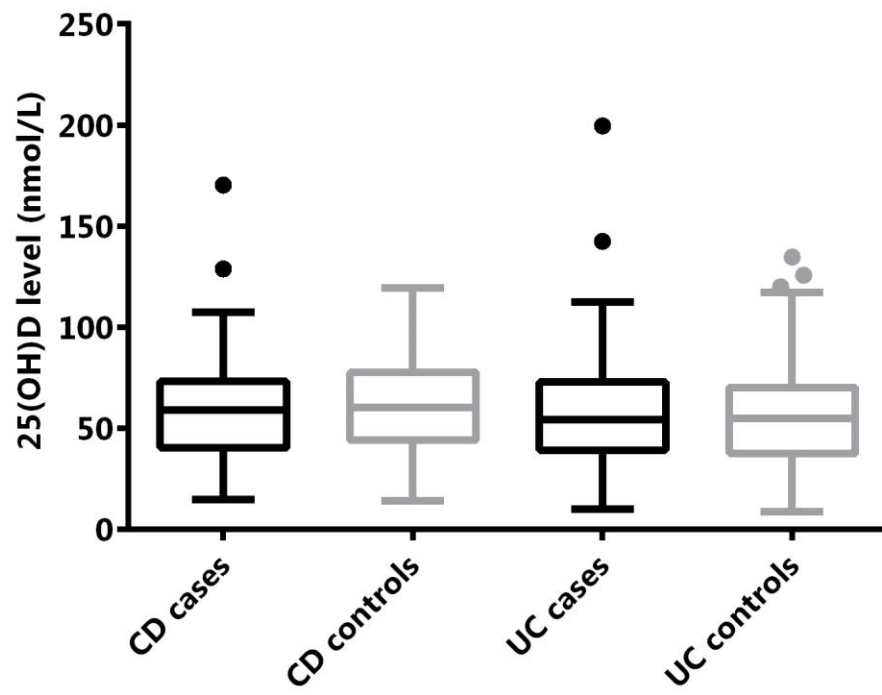
CI: confidence interval; OR: odds ratio

* adjusted for smoking status

Figure legends

Figure 1. Distribution of serum 25(OH)D concentrations of inflammatory bowel disease cases and their controls

25(OH)D: 25-hydroxyvitamin D; CD: Crohn's disease; UC: ulcerative colitis



Supplementary Table 1. Odds of Crohn's disease and ulcerative colitis according to 25(OH)D levels and dietary vitamin D intake excluding cases diagnosed less than 18 months after enrolment and their controls

	Cases, n (%)	Controls, n (%)	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Crohn's disease				
Quartile of 25(OH)D				
1 (≤ 44.0 nmol/L)	18 (30.0)	32 (26.7)	1.00	1.00
2 (44.1-60.3 nmol/L)	18 (30.0)	31 (25.8)	1.01 (0.46-2.20)	0.91 (0.40-2.05)
3 (60.4-77.5 nmol/L)	14 (23.3)	28 (23.3)	0.90 (0.37-2.15)	0.75 (0.30-1.88)
4 (≥ 77.6 nmol/L)	10 (16.7)	29 (24.2)	0.61 (0.24-1.55)	0.59 (0.23-1.51)
			$p_{\text{trend}} = 0.28$	$p_{\text{trend}} = 0.24$
Quartile of dietary vitamin D				
1 (≤ 2.21 $\mu\text{g/day}$)	19 (31.7)	30 (25.0)	1.00	1.00
2 (2.22-3.03 $\mu\text{g/day}$)	6 (10.0)	33 (27.5)	0.31 (0.11-0.88)	0.35 (0.12-1.04)
3 (3.04-4.10 $\mu\text{g/day}$)	15 (25.0)	25 (20.8)	0.95 (0.39-2.32)	1.20 (0.47-3.08)
4 (≥ 4.11 $\mu\text{g/day}$)	20 (33.3)	32 (26.7)	1.09 (0.39-3.02)	1.24 (0.43-3.57)
			$p_{\text{trend}} = 0.36$	$p_{\text{trend}} = 0.23$

Supplementary Table 1. (continued)

	Cases, n (%)	Controls, n (%)	Unadjusted OR (95% CI)	Adjusted OR* (95% CI)
Ulcerative colitis				
Quartile of 25(OH)D				
1 (≤ 37.5 nmol/L)	29 (19.5)	74 (24.8)	1.00	1.00
2 (37.6-54.9 nmol/L)	43 (28.9)	73 (24.5)	1.48 (0.84-2.61)	1.50 (0.84-2.69)
3 (55.0-70.1 nmol/L)	32 (21.5)	73 (24.5)	1.16 (0.62-2.16)	1.27 (0.66-2.43)
4 (≥ 70.2 nmol/L)	45 (30.2)	78 (26.2)	1.49 (0.81-2.72)	1.36 (0.72-2.59)
			$p_{\text{trend}} = 0.29$	$p_{\text{trend}} = 0.48$
Quartile of dietary vitamin D				
1 (≤ 1.94 $\mu\text{g/day}$)	28 (18.8)	72 (24.2)	1.00	1.00
2 (1.95-3.09 $\mu\text{g/day}$)	49 (32.9)	73 (24.5)	1.71 (0.96-3.05)	1.70 (0.93-3.09)
3 (3.10-4.50 $\mu\text{g/day}$)	43 (28.9)	74 (24.8)	1.50 (0.78-2.87)	1.52 (0.78-2.96)
4 (≥ 4.51 $\mu\text{g/day}$)	28 (18.8)	76 (25.5)	0.91 (0.44-1.86)	0.89 (0.43-1.85)
			$p_{\text{trend}} = 0.39$	$p_{\text{trend}} = 0.37$

25(OH)D: 25-hydroxyvitamin D; CI: confidence interval; OR: odds ratio

* adjusted for smoking status