



Active Crohn's disease is associated with low vitamin D levels



Søren Peter Jørgensen ^{a,*}, Christian Lodberg Hvas ^a,
Jørgen Agnholt ^a, Lisbet Ambrosius Christensen ^a,
Lene Heickendorff ^b, Jens Frederik Dahlerup ^a

^a Department of Medicine V, Hepatology and Gastroenterology, Aarhus University Hospital, Denmark

^b Department of Clinical Biochemistry, Aarhus University Hospital, Denmark

Received 24 August 2012; received in revised form 13 December 2012; accepted 14 January 2013

KEYWORDS

Crohn's disease;
Vitamin D;
Smoking;

Abstract

Background and aims: Crohn's disease prevalence increases with increasing latitude. Because most vitamin D comes from sunlight exposure and murine models of intestinal inflammation have demonstrated beneficial effects of 1,25-(OH)₂ vitamin D treatment, we hypothesised that Crohn's disease activity is associated with low vitamin D levels.

Methods: In a cross-sectional study of 182 CD patients and 62 healthy controls, we measured serum 25-OH vitamin D. Stratified analysis was used to compare 25-OH vitamin D levels with Crohn's disease activity index, C-reactive protein, smoking status, intake of oral vitamin D supplements and seasonal variation in CD patients and healthy controls.

Results: Serum 25-OH vitamin D was inversely associated with disease activity: Median 25-OH vitamin D levels of Crohn's disease in remission, mildly, and moderately active diseases evaluated by Crohn's disease activity index were 64, 49, and 21 nmol/l ($p < 0.01$) and by CRP 68, 76, and 35 nmol/l ($p < 0.05$), respectively. Patients who took oral vitamin D supplementation had lower Crohn's disease activity index ($p < 0.05$) and C-reactive protein ($p = 0.07$) than non-users. Crohn's disease patients who smoked had lower vitamin D levels (51 nmol/l) than patients who did not smoke (76 nmol/l), $p < 0.01$. Overall, Crohn's disease patients did not differ from healthy controls regarding 25-OH vitamin D levels.

Conclusions: Active Crohn's disease was associated with low serum 25-OH vitamin D. Patients who smoked had lower 25-OH vitamin D levels than patients who did not smoke, independently of disease activity.

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Abbreviations: CD, Crohn's disease; 25OHD, 25-OH vitamin D; 1,25(OH)₂D, 1,25-(OH)₂ vitamin D; VDR, vitamin D receptor; TNF- α , tumour necrosis factor alpha; INF- γ , interferon gamma; (TGF)- β , transforming growth factor; CRP, C-reactive protein; CDAI, Crohn's disease activity index.

* Corresponding author at: Department of Medicine V, Hepatology and Gastroenterology, Aarhus University Hospital, Nørrebrogade 44, DK-8000 Aarhus C, Denmark. Tel.: +45 8949 2899; fax: +45 8949 2740.

E-mail addresses: soerjoer@rm.dk (S.P. Jørgensen), chrishva@rm.dk (C.L. Hvas), jorgen.agnholt@aarhus.rm.dk (J. Agnholt), lisbchte@rm.dk (L.A. Christensen), leneheic@rm.dk (L. Heickendorff), jensdahl@rm.dk (J.F. Dahlerup).

1. Introduction

In Crohn's disease (CD), an inflammatory bowel disease, tolerance towards the commensal intestinal flora is broken.¹ Vitamin D may serve as a co-regulator involved in a balanced immune response, besides the effects on bone homeostasis.² The main source of vitamin D in humans is cutaneous biosynthesis upon sunlight exposure.³ CD patients have lower vitamin D levels than healthy individuals, and CD prevalence increases with increasing latitude.^{4–8} Time of day, season and latitude all influence the zenith angle of the sun. Most people rely on casual exposure to sunlight to satisfy their vitamin D requirement, and therefore 25-OH vitamin D (25OHD) levels exhibit seasonal variation.³ It has been hypothesised that low sunlight exposure and hence low vitamin D levels may be involved in CD pathogenesis. Murine – and in vitro studies – support this hypothesis.^{9–11} One clinical trial has tested vitamin D compared to placebo as adjuvant therapy in CD and found non-significantly lower relapse rate in the vitamin D group.¹²

Following ingestion or synthesis in the skin, vitamin D is hydroxylated by the liver to the storage-type 25OHD. Final activation takes place in the kidneys by hydroxylation to the active metabolite 1,25-(OH)₂ vitamin D (1,25(OH)₂D). In addition to renal hydroxylation, 25OHD is 1- α hydroxylated in dendritic cells and macrophages.¹³ Vitamin D exerts its effects via the intracellular vitamin D receptor (VDR), which is activated by 1,25(OH)₂D. Immune system express VDR, in particular activated T lymphocytes and dendritic cells.¹⁴ These cells are key mediators of inflammation in CD, and bacterial antigen-stimulation of dendritic cells results in an inflammatory process carried out by activated T lymphocytes.¹⁵ This process is regulated by vitamin D and 1,25(OH)₂D. In vitro studies using dendritic cells and T lymphocytes for experimental inflammation show a decrease in the pro-inflammatory cytokines tumour necrosis factor alpha (TNF- α) and interferon gamma (INF- γ) following 1,25(OH)₂D addition.^{11,16} Furthermore, 1,25(OH)₂D increases the anti-inflammatory cytokines interleukin (IL)-10 in human T cells and transforming growth factor (TGF)- β in murine T cells in vitro.^{17,18} Based on these results we hypothesised that vitamin D may affect the inflammatory reaction in CD.

Our aim was to investigate whether active CD was associated with low serum levels of 25OHD, and to compare 25OHD levels in CD patients with healthy individuals in a cross-sectional design. Furthermore, we aimed to investigate whether disease behaviour, body mass index, and smoking habits influenced 25OHD levels in CD.

2. Material and methods

2.1. Patients and controls

During January 2000 through April 2007, we collected data and biobank material (serum) from CD patient visiting our outpatient clinic for future research purpose. December 2008 our biobank was scrutinised and serum from 182 CD patients collected over these years was available for analysis. Besides the 182 CD patients (105 women and 77 men), 62 healthy individuals (32 women and 30 men) were used in this study. Median ages for CD patients and healthy controls were 36 and 32 years, respectively (Table 1).

Inclusion criteria for CD patients were verified CD based on clinical, radiological, and histopathological criteria. There were no exclusion criteria, although in sub-analysis we excluded patients missing the specific data which was analysed. Healthy controls were hospital personnel randomly asked to participate in the period, June 2005 to June 2006. Exclusion criteria for the healthy controls were health issues that had resulted in a consultation with a physician within the past year prior to inclusion. For CD patients, the following biochemical and clinical parameters were recorded: C-reactive protein (CRP) and Crohn's disease activity index (CDAI) (Table 2). In all participants 25OHD, age, gender, weight, height, smoking status and vitamin D supplement use were recorded. Vitamin D supplementation was defined as an oral intake of 400 international units (IU) or more per day. Since 1997 at department V, we have recommended vitamin D 800 IU daily to CD patients who use corticosteroids. No persons in the study were recorded to ingest more than 800 IU of vitamin D per day. Complete data was obtained with regard to 25OHD, gender, and age. Each of the other clinical parameters used had missing values. Patients and controls were withdrawn from the analyses in case of missing values regarding the specific hypothesis tested. For that reason, none of the sub-analyses included all participants, and the number of individuals included in the specific analysis is provided in each figure. The study was approved by the Ethics Committee of the County of Aarhus, Denmark (j. no. 1998/4330). All patients gave written consent to participation.

2.2. Definitions of disease activity and vitamin D replete

Disease activity was evaluated by the combined para-clinical and clinical symptom scores (CDAI) and the biochemical inflammation parameter (CRP), respectively. A CDAI of less than 150 defines remission, $150 \leq \text{CDAI} < 220$ defines mild disease activity, and $\text{CDAI} \geq 220$ defines moderate disease activity.¹⁹ A CRP below 75 nmol/l (~8 mg/l) is within a

Table 1 Characteristics in 182 CD patients compared with 62 healthy controls (SD: standard deviation; IQR: interquartile range).

	Controls	CD	p
N	62	182	–
Age (median and (range))	32 (18–62)	36 (17–78)	–
Females (%)	52	57	–
Overall serum-25OHD, nmol/l (median)	65 (n=62), SD:25, IQR:47–82	69 (n=182), SD:33, IQR:39–88	NS
Serum-25OHD, nmol/l (median) (non-supplemented)	65 (n=43), SD:25, IQR:47–85	61 (n=46), SD:35, IQR:32–86	NS
Vitamin D supplement users if status known (%)	10 (n=5 of 48)	45 (n=38 of 84)	p<0.01

Table 2 Characteristics of CD patients with CDAI and/or CRP registered.

	CDAI remission	CDAI relapse	CRP remission	CRP relapse
N	102	12	103	37
Age in years (median and (range))	34 (18–66)	29 (21–51)	35 (18–35)	31 (20–72)
Females (%)	60	66	65	40
Serum-albumin, ($\mu\text{mol/l}$) (median and interquartiles)	645 (616–675)	541(488–578)	642 (613–672)	582 (523–671)
Ileal disease (%)	10	–	17	13
Frequency of patients in high25OHD-level season (%)	35	41	42	48
Frequency of smokers	34	70	36	36

normal range in our hospital laboratory. We defined a normal CRP as CD in remission. Additionally, we grouped CRP in mild disease (CRP 75–150 mol/l) and moderate to severe disease (CRP \geq 150 nmol/l).

Total 25OHD level is estimated by the addition of serum 25OHD₂ and serum 25OHD₃. Body stores of vitamin D are reflected by serum-levels of 25OHD, and current recommendations are based on bone homeostasis. Normal vitamin D status is defined by the lowest 25OHD level needed to avoid secondary hyperparathyroidism, and when this level is reached, the person is defined as vitamin D replete.²⁰ The definition of this optimum is debated.²⁰ The most conservative definition of vitamin D replete and vitamin D deficiency is more than and less than 50 nmol/l serum 25OHD, respectively.²¹

2.3. Blood sampling and vitamin D analyses

Blood samples were drawn from non-fasting individuals between 8 AM and 14 PM. Serum was harvested following centrifugation and cryopreserved at $-80\text{ }^{\circ}\text{C}$ within 1–4 h after blood sample collection and stored until analysis. Serum 25OHD₂ and 25OHD₃ were analysed by isotope-dilution liquid chromatography–tandem mass spectrometry on an API300 TM mass spectrometer (Applied Biosystems, Lincoln, U.S.A.) by a method adapted from Maunsell et al.²² The interassay coefficients of variation (CVs) for serum 25OHD₂ were 8.5% at 23.4 nmol/l and 8.0% at 64.4 nmol/l, and for serum 25OHD₃ 9.6% at 24.8 nmol/l and 8.1% at 47.7 nmol/l. Total 25OHD is estimated by the addition of 25OHD₂ and 25OHD₃. C-reactive protein was measured as part of a routine disease evaluation.

2.4. Statistics

Non-parametric statistical tests were applied in bivariate comparisons (Mann–Whitney *U* test) and comparisons across categories of independent samples (Kruskall–Wallis test and Spearman's rho). Fischer's exact test was used to evaluate differences in vitamin D supplementation use. Test for confounding was carried out using a separate analysis of stratified data and by inserting potential confounders into a linear multiple regression model. All statistical analyses were performed using STATA version 9.2 software (www.stata.com). A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Disease activity is inversely correlated with vitamin D levels

Disease activity, evaluated by CDAI and CRP, was inversely correlated with 25OHD levels. Thus, CD patients with active disease had lower levels of 25OHD than patients in remission (Fig. 1). In CD patients with CDAI less than 150 which indicates clinical remission, the median serum level of 25OHD was 64 nmol/l whereas the level seen in mild disease was 49 nmol/l and 21 nmol/l in moderately active disease, respectively (Kruskall–Wallis test and Spearman's rho, $p < 0.01$). With regard to CRP as a marker of inflammation, we observed the same association with a serum 25OHD level of 68 nmol/l in CD patients in remission compared with 74 nmol/l in patients with slightly elevated CRP and 35 nmol/l in patients with moderately elevated CRP (Kruskall–Wallis test, $p < 0.05$). The linear trend across the three CRP categories was not as strong as for CDAI (Spearman's rho, $p = 0.12$).

In order to take into account oral vitamin D supplementation, patients were stratified into patients who used vitamin D supplementation and patients registered as either non-users or as with user status "unknown". Statistically significantly lower CRP levels were observed among the vitamin D supplement users (Mann–Whitney *U* test, $p < 0.01$) (Fig. 2). The same tendency was seen regarding CDAI, with an overall lower median level among the supplement users, although these data did not reach statistical significance (Mann–Whitney *U* test, $p = 0.07$) (Fig. 2).

3.2. Patients who smoke have lower vitamin D levels than patients who are non-smokers

Smoking increases the risk of developing CD, and in established CD the risk of having a relapse is increased amongst smokers.²³ In CD patients registered as smokers, we observed a statistically significantly lower median 25OHD level (51 nmol/l) than in CD patients registered as non-smokers (76 nmol/l) (Mann–Whitney *U* test, $p < 0.01$). This difference was even more pronounced when registered vitamin D supplement users were removed from data analysis: Smokers had a 25OHD level of 43 nmol/l compared with 74 nmol/l in non-smokers (Mann–Whitney *U* test, $p < 0.001$) (Fig. 3).

3.3. Smoking did not confound the association between disease activity and 25OHD

Because smoking may affect disease activity and smoking was related to low 25OHD levels, the distribution of smokers could confound the association between disease activity and 25OHD levels. In order to identify any such confounding, we carried out an analysis following stratification by smoking habits and by inserting smoking into a linear regression model. None of these tests changed the association between disease activity and 25OHD, and the association was similar among smokers and non-smokers (data not shown). We therefore conclude that disease activity and smoking are both independently associated with low 25OHD in CD.

Other potential risk factors for low vitamin D levels include body mass index (BMI) and localisation of disease. In CD patients, BMI was categorised into <20, 20 to 24, and >24 kg/m.² We observed no difference in 25OHD levels between these groups (data not shown). Localisation of disease was categorised according to the Vienna criteria into ileal, ileo-colonic, and colonic diseases, and no significant difference was observed (data not shown). We also examined patients who had undergone bowel surgery and found no difference in 25OHD levels compared with non-operated patients (data not shown).

Data on smoking habits was missing in 14 patients (8%), CDAI was missing in 68 patients (37%), and CRP was missing in 42 patients (23%). We analysed cases with missing values separately to identify potential selection bias. In cases with missing CDAI or missing CRP, we observed the same association between smokers and non-smokers (data not shown), and in cases with missing information on smoking, we observed lower 25OHD levels in patients with active disease (data not shown). We therefore conclude that missing data did not introduce bias.

3.4. Seasonal variation in 25OHD may be outweighed by vitamin D supplement use

In CD patients who used vitamin D supplements, 25OHD level was statistically significantly higher (77 nmol/l) than among non-users (55 nmol/l), Mann–Whitney *U* test, $p < 0.01$. We examined the variation in 25OHD levels between high-level season (May through October) and low-level season (November through April). We found that in the low-level season, CD patients reported to ingest vitamin D supplements (400–800 IE) and had significantly higher levels of 25OHD (77 nmol/l) than non-users (44 nmol/l) (Mann–Whitney *U* test, $p < 0.001$) (Fig. 4). In the high-level season, no statistically significant difference was observed between supplement users (80 nmol/l) and non-users (86 nmol/l). Our data indicate that vitamin D supplementation may raise 25OHD levels to high-level season levels during the low-level season in CD patients.

Overall, CD patients had 25OHD levels comparable to those in healthy controls ($p = 0.95$). However, we found that vitamin D supplementation was more frequent in CD patients (44%) than in controls (10%) (Mann–Whitney *U* test, $p < 0.05$). We therefore examined the difference in 25OHD levels between CD patients and healthy controls when only analysing vitamin D supplement non-users. Still, we were unable to detect any statistically significant difference in 25OHD levels between CD patients overall and healthy volunteers.

4. Discussion

The main finding in this study is that disease activity in CD is associated with low levels of 25OHD. Disease activity was evaluated using both the clinical one-week questionnaire-based CDAI and CRP which is a biochemical marker of

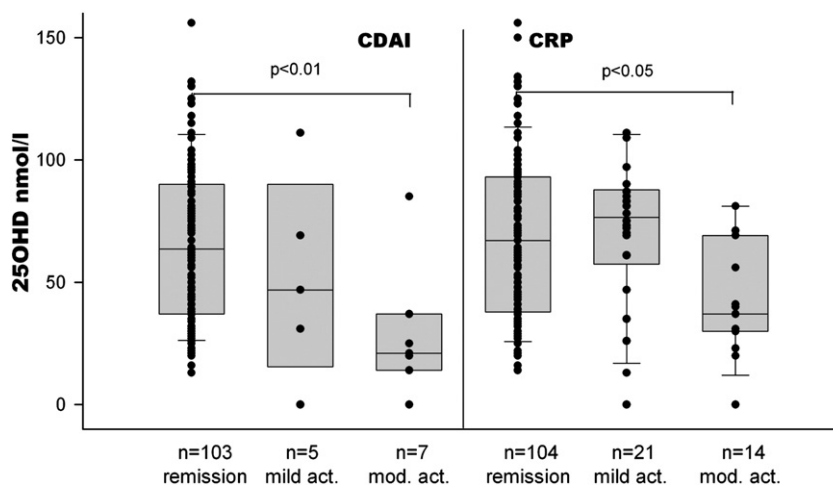


Figure 1 Disease activity in Crohn's disease was inversely associated with serum 25OHD. The strongest association was found for CDAI with a p -value of 0.01 (Kruskal–Wallis). As for CRP, we observed a slight increase of 25OHD in the group with mild disease activity (mild act.), but as a decrease was observed in the group with moderate activity (mod. act.), an overall significant decrease was seen, $p < 0.05$, Kruskal–Wallis. Median, 95th and 5th percentiles are illustrated.

inflammation. While the association between CDAI and 25OHD was strong, there was no falling trend across the categories for CRP. We wished to investigate association between 25OHD levels and a marker of inflammation because vitamin D may affect the inflammatory process directly. Although CRP may not differentiate accurately between relapse (mild disease) and remission, it may be used as a measure of disease activity in terms of inflammation. In order to distinguish between patients with no or mild disease (slightly elevated CRP) and patients with moderate disease, we grouped patients arbitrarily into slightly and moderately elevated CRP. Furthermore, clinical activity with isolated mucosal inflammation could be misclassified as remission due to normal CRP. This may in part explain the lack of a falling trend across CRP categories. Future studies should include mucosal markers of inflammation, e.g. faecal calprotectin, in order to further detail this association.

Our findings lead to the question whether vitamin D supplementation is beneficial in Crohn's disease. Some patients in the study received oral vitamin D supplementation, but because treatment was not randomised and data were not prospectively collected before and after treatment, we are unable to conclude if raising serum 25OHD levels reduced the risk of disease relapse. Instead, we examined whether median levels of CDAI and CRP were related to prevalent vitamin D supplementation in this cross-sectional design. We observed lower CDAIs and CRP levels in CD patients using vitamin D supplementation than in non-users. This may represent a healthy-patient effect, i.e. that patients are in remission due to a high compliance to medical therapies including vitamin supplementation, or it may reflect a beneficial effect of vitamin D supplementation on the disease course.

Another finding is that CD patients who smoked had lower 25OHD levels than CD patients who did not smoke. When examining vitamin D supplement non-users only, the difference was even more pronounced. Low levels of 25OHD in smokers without CD have been reported earlier.^{24,25} Smoking is one of the most well-characterised predisposing factors to

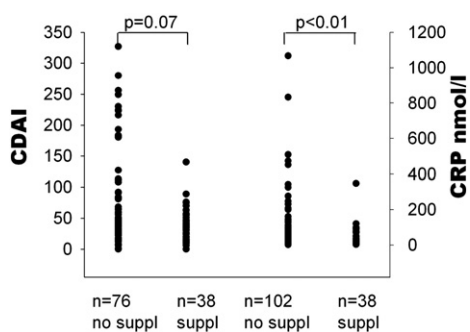


Figure 2 Disease activity in CD estimated by CDAI and CRP and stratified by vitamin D supplement use (suppl) and vitamin D supplement non-use or unknown (no suppl). Crohn's disease activity index was non-significantly lower in supplement users (median-value: 35) than in non-users (median-value: 44), Mann–Whitney U test, $p=0.07$. CRP was statistically significantly lower in supplement users (median-value: 21 nmol/l) than in non-users (median-value: 48 nmol/l), Mann–Whitney U test, $p<0.01$.

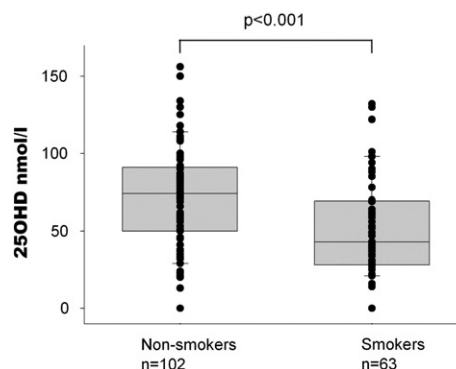


Figure 3 Crohn's disease patients registered as smokers have statistically significantly lower 25OHD levels than CD patients registered as non-smokers (Mann–Whitney U test, $p<0.001$). Median, 95th and 5th percentiles are illustrated.

increase disease activity in CD.²³ For that reason smoking had the potential to be a confounder, and we performed stratified analyses, which showed that both smoking and disease activity were independently associated with low levels of 25OHD.

Our data are limited by missing values which are reflected by a reduced number of individuals included in the analyses because cases with missing values were excluded from the analysis. For this to introduce bias, cases with missing values would have to have a particular distribution of 25OHD. We find it likely that factors which could increase serum 25OHD such as fat fish or extensive sun exposure are not related to disease activity, although one may speculate that patients with active disease could spend more time indoors and thus have lower sun exposure than patients in remission. In this study we did not have data on level of sun-exposure and are unable to analyse this matter. Because no food items in Denmark are enriched with vitamin D, vitamin D occurs naturally in only few food items, and relatively short sun exposure is necessary to ensure normal serum 25OHD, we find that the risk of such bias is low. Even though, this study is limited by the cross-sectional design, and for that reason our findings of low vitamin D in CD patients with active disease could reflect other causal explanations. Lower physical activity, which is probably associated with lower sun-exposure, and difference in food intake in CD-patients with active disease, are alternative explanations of our finding.

Vitamin D levels fluctuate with the season and in Denmark, the lowest 25OHD levels are observed from November to April.²⁶ For this reason, the 25OHD levels were also analysed according to whether it fell in one of the two groups: "high 25OHD level season" (May–October) or "low-25OHD-level season" (November–April). In line with this, we observed a clear seasonal difference in 25OHD levels. Our data indicate that vitamin D supplementation may equalise this unbalance. We observed similar 25OHD levels in supplement users in the low level season compared with non-users and users in the high level season. Contrary to recent reports, we did not find lower overall 25OHD levels in CD compared with healthy controls.^{4–6} A possible explanation is that low levels of 25OHD are found in a sub-group of patients, e.g. patients with active disease.

The term vitamin D replete is based on maximally suppressed levels of parathyroid hormone. The 50 nmol/l

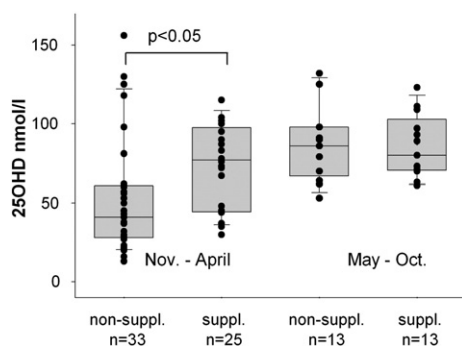


Figure 4 Serum levels of 25OHD are significantly higher in vitamin D supplemented CD patients in the 25OHD low-level season compared with vitamin D supplement non-users (Mann–Whitney *U* test, $p < 0.001$) In the high-level 25OHD season, no difference is observed between vitamin D supplement users and non-users. Median, 95th and 5th percentiles are illustrated.

level of 25OHD used to define the threshold for vitamin D deficiency is a minimum level. Consensus on the setting of this threshold has not been reached. Some investigators set the lower limit at 80 nmol/L.²⁰ In the past decade, vitamin D deficiency has been associated with an increased risks of cancers, cardiovascular disease and autoimmune diseases.³ However, the optimal level of 25OHD has not been related to these conditions. The 25OHD level could be increased to 220 nmol/L or more without increased risk of vitamin D intoxication.²⁷

In conclusion, our data indicate that low 25OHD may be more frequent in CD patients with active disease, and that CD patients who smoke have low 25OHD levels. We are aware of the limitations in our study. But still, we hypothesise that vitamin D causally influences disease course in CD and propose this matter to be further investigated in clinical trials. One randomised placebo controlled trial has been published and found insignificantly lower relapse in CD compared with placebo.¹²

Funding

This study was supported by grants of Hørslev Foundation, Denmark.

Contribution

Authors: Søren Peter Jørgensen (SPJ), Christian Lodberg Hvas (CH), Jørgen Agnholt (JA), Lisbet Ambrosius Christensen (LAC), Lene Heckendorff (LH), Jens Frederik Dahlerup (JFD), SPJ, JA and JFD contributed to the design of the study. All authors contributed in data collection. SPJ, JA, CH, and JFD participated in data analysis. All authors contributed to the writing and revision of the manuscript. All authors saw and approved the final version of the manuscript.

Conflict of interest

None to declare.

Acknowledgements

We thank Rikke Andersen for the technical assistance.

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