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Vitamin D metabolites are lower with active Crohn's disease and spontaneously recover with development of remission

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Abstract

Background: Vitamin D deficiency is associated with active Crohn's disease (CD). However, it remains unclear if lower 25-hydroxyvitamin D [25(OH)D] concentration is the cause, or consequence, of intestinal inflammation. Existing literature has focused on circulating 25(OH)D rather than the active metabolite 1,25$(OH)_2$D, or its breakdown product, 24,25$(OH)_2$D. We aimed to characterise vitamin D metabolism in a cohort of patients with active and inactive CD.

Methods: Fifty-four patients with CD and not on corticosteroids or vitamin D supplements, were enrolled in a 6-month prospective cohort study. Sera were collected on enrolment and at 6 months and tested for 25(OH)D, 1,25$(OH)_2$D, 24,25$(OH)_2$D using liquid chromatography tandem mass spectroscopy as well as vitamin-D-binding protein.

Results: There were no differences in 25(OH)D or 1,25$(OH)_2$D levels between participants with active *versus* inactive disease. Levels of 24,25$(OH)_2$D were significantly lower in those with active compared with inactive disease (mean 3.9 *versus* 6.0 µmol/l; $p = 0.007$) and therefore the ratio of 25(OH)D:24,25$(OH)_2$D was higher (mean 17.3 *versus* 11.1; $p = 0.001$). In those patients with active disease who achieved remission, there was a mean increase in 25(OH)D of 32.3 nmol/l (i.e. to a level in the sufficient range) and 24,25$(OH)_2$D of 2.1 µmol/l. These increases were not seen in patients with persistently active or inactive disease.

Conclusion: Levels of 24,25$(OH)_2$D, but not 25(OH)D, were lower in patients with active CD, and spontaneously increased with resolution of underlying inflammation. The utility of 24,25$(OH)_2$D as a biomarker of disease activity and vitamin D status in CD warrants further exploration.

Keywords: Crohn's disease, inflammatory bowel disease, vitamin D

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Introduction

Active Crohn's disease (CD) and ulcerative colitis (UC) are associated with vitamin D deficiency in many[1–3] but not all studies.[4,5] Vitamin D deficiency is defined by a 25-hydroxyvitamin D [25(OH)D] concentration in serum, or plasma, cut-off of <50 nmol/l.[6,7] There are a number of factors that could account for the association between vitamin D deficiency and active inflammatory bowel disease (IBD) such as corticosteroid use, reduced sunlight exposure and reduced vitamin D oral intake or absorption.[8]

Vitamin D is acquired from diet, or through dermal synthesis, predominantly in the form of vitamin D_3. Vitamin D_2 is produced by ultraviolet (UV) irradiation of ergosterols in plants but contributes modestly to the total circulating vitamin D, except in populations with vitamin-D_2-supplemented food. Vitamins D_2 and D_3 undergo comparable metabolism, so when vitamin D_3 metabolism is referred to, it also applies to vitamin D_2 metabolites. Vitamin D_3 undergoes 25-hydroxylation in the liver by the cytochrome p450 enzyme, CYP2R1, to produce 25$(OH)D_3$,

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which has little biological activity. The majority (85–90%) of $25(OH)D_3$ circulates tightly bound to the vitamin-D-binding protein (VDBP).[9] The remaining non-VDBP-bound fraction (bioavailable $25(OH)D_3$) is bound to albumin with less than 1% of $25(OH)D_3$ unbound or free. It has been proposed that the free, or bioavailable, $25(OH)D_3$ may be more physiologically relevant than total $25(OH)D_3$, although this has not been formally evaluated.[9,10] Circulating $25(OH)D_3$ is further hydroxylated in the kidney and extra-renal tissues by the 1α-hydroxylase CYP27B1 to produce the metabolically active $1,25(OH)_2D_3$. $1,25(OH)_2D_3$ exerts its actions principally through binding to a nuclear vitamin D receptor. Changing concentrations of $1,25(OH)_2D_3$ and $25(OH)D_3$ modulate the expression of the *CYP24A1* which is responsible for the production of 24,25-dihydroxy vitamin D ($24,25(OH)_2D_3$), $1\alpha,25(OH)_2D_3$-26,23S-lactone and calcitroic acid (1α-hydroxy-23-carboxy-24,25,26,27-tetranorvitamin D_3). While 24-hydroxylase CYP24A1 is most abundant in the proximal and distal tubules of the kidney, it has also been detected in essentially all vitamin D target tissues including the colon.

The association between active IBD and vitamin D deficiency has led investigators to study vitamin D supplementation as a treatment for IBD in a series of small studies that have yielded mixed results.[11–13] In the largest study, a randomized, double-blind placebo-controlled trial, 94 CD patients in remission were randomized to receive either 1200 IU vitamin D_3, or placebo, once daily for 1 year. Vitamin D supplementation reduced the rate of clinical relapses from 29% to 13% ($p = 0.06$).[11] A further study in UC supplementing participants with 40,000 IU vitamin D_3 for 8 weeks, using an inactive UC and non-IBD control groups, found a reduction in faecal calprotectin and simple clinical colitis score, but not the partial Mayo score.[12] However, a pilot randomized open-label study treated 124 UC and CD patients with either 150,000 IU vitamin D_3 daily, elemental calcium or no treatment and showed no improvement in disease activity scores with vitamin D supplementation.[13]

Our group has recently shown in a mouse model, that circulating $25(OH)D_3$ and the active metabolite $1,25(OH)_2D_3$ acutely drop after inducing colitis with dextran sodium sulphate, associated with an increase in gene expression of the *CYP24A1*.[14] Further, in a large Australian cohort of patients with CD in steroid-free remission, a low 25(OH)D level did not predict subsequent relapse which would otherwise be expected if vitamin D deficiency predisposed to intestinal inflammation.[15]

We hypothesized that the circulating serum $25(OH)D_3$ concentrations will be lower in patients with active CD as a result of active catabolism of $25(OH)D_3$ to downstream metabolites, such as $24,25(OH)_2D_3$. Further, we hypothesized that these changes will reverse with resolution of inflammation.

The aim of this study was to characterise the effect of intestinal inflammation on the metabolism of vitamin D, by examining a range of vitamin D metabolites in the setting of active and inactive CD.

Material and methods

Patients and design

Patients with CD were prospectively recruited from the IBD clinic at St. Vincent's Hospital, Sydney, Australia, between March and June 2017. All patients were diagnosed with CD according to standard clinical, endoscopic, and radiological criteria[16] and were phenotyped according to the 'Montreal Classification'. Patients were aged between 16 and 60 years and had either colonic (Montreal L2) or ileocolonic (Montreal L3) disease. We included two groups of patients; those with moderate–severe disease activity and those in remission. Moderate–severe disease activity was defined by a CD activity index (CDAI) ≥ 220 in addition to an objective marker of active inflammation [C-reactive protein (CRP) ≥ 10 mg/l, faecal calprotectin > 250 µg/g or active ulceration seen at ileocolonoscopy within 3 months]. Inactive disease was defined by CDAI < 150 and either CRP < 10 mg/l, faecal calprotectin < 150 µg/g; or no ulceration at ileocolonoscopy within 3 months. Patients were excluded if they were on vitamin D supplements or corticosteroids within 4 weeks of recruitment, if they were pregnant, had short bowel syndrome, isolated small bowel CD or remission that was induced by colonic resection.

At enrolment, baseline data, including demographics, disease and medication history were collected. Participants completed diet and

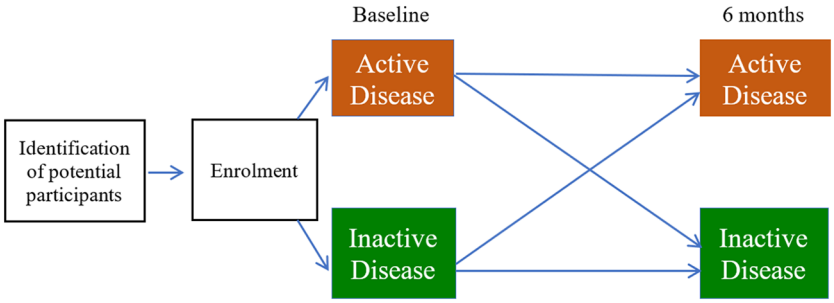

Figure 1. Study design.
Participants with active or inactive CD were enrolled. Active disease was defined by CDAI ⩾ 220, in addition to an objective marker of active inflammation (CRP ⩾ 10 mg/l, faecal calprotectin > 250 µg/g or active ulceration seen at ileocolonoscopy within 3 months). Inactive disease was defined by CDAI < 150 and either CRP < 10 mg/l, faecal calprotectin < 150 µg/g or no ulceration at ileocolonoscopy within 3 months. Cross-sectional analysis of 25(OH)D_3, 24,25$(OH)_2D_3$, 1,25$(OH)_2D_3$ and VDBP was performed at baseline. Participants were followed up for 6 months, with longitudinal analysis of vitamin D metabolites. CD, Crohn's disease; CDAI, CD activity index; CRP, C-reactive protein; 25(OH)D, 25-hydroxy vitamin D, VDBP, vitamin-D-binding protein.

sunlight questionnaires at baseline and at 6 months. Blood was collected, and serum stored from all participants at enrolment and after 6 months (Figure 1). Vitamin D metabolite testing was performed after the study period, effectively blinding treating physicians to the vitamin D results.

Participants were followed for a period of 6 months. Treatment of CD during the study period, including the use of corticosteroids, was left to the discretion of the treating physician and recorded in the study records. If patients commenced on vitamin D supplementation during the study period, then they were not included in the follow-up analysis. Disease relapse was defined as greater than a 100-point rise in CDAI to at least 150 with associated objective markers of relapse (CRP ⩾ 10 mg/l, faecal calprotectin > 250 µg/g or active ulceration seen at ileocolonoscopy). Development of remission from active disease was defined as a CDAI < 150 and either CRP < 10 mg/l, faecal calprotectin < 150 µg/g or no ulceration at ileocolonoscopy.

Peripheral blood was collected by venepuncture. Routine laboratory haematology and biochemistry tests were performed immediately, and a serum sample of 1–1.5 ml was stored at −20°C for later analysis of vitamin D metabolites.

Biochemical measurements

Concentrations of serum 25(OH)D_3 were measured using liquid chromatography tandem mass spectroscopy (LC/MS/MS) at Metabolomics Australia, University of Western Australia, using two Agilent 1290 UPLC binary pumps coupled to an Agilent 6460 triple quadrupole tandem mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). This assay is certified by the Centre for Disease Control (National Institutes of Health, USA) for both precision and accuracy of 25(OH)D_3 measurement with a correlation of R^2 0.9979.[17] The 24,25$(OH)_2D_3$ and 1,25$(OH)_2D_3$ were assayed on the same LC/MS/MS, after sample derivatization with triazolinedione reagents.[18] VDBP was measured by immunonephelometry (Dade Behring, Liederbach, Germany; interassay coefficients of variability (CV) < 6%), and albumin was measured by dye-binding reaction to bromocresol green (interassay CV < 5%). Free and bioavailable (free plus albumin-bound) concentrations of 25(OH)D were calculated using VDBP and albumin concentrations, according to modified 'Vermeulen' equations previously published and validated.[19] Albumin, CRP, white cell count and platelet count were analysed using routine laboratory techniques.

Questionnaires

Within 2 weeks of the baseline blood draw, participants completed a baseline questionnaire (Supplementary Data Content 1) including details of their demographics, ethnicity, smoking history, alcohol consumption, medication use, supplements, CD diagnosis and treatment. We used validated questions to measure sunlight exposure and dietary vitamin D intake.[20] Sunlight exposure

assessment included questions related to skin colour and tanning characteristics, time spent outdoors between the hours of 10 am and 3 pm in the preceding month on weekdays and weekends, area of skin exposed and use of sunscreen. Vitamin D intake was measured using a food frequency questionnaire of foods with highest vitamin D content. At study exit, a follow-up questionnaire (Supplementary Data Content 1) was administered including details of symptoms, medications, smoking, sun exposure and dietary intake.

Data on ambient levels of ultraviolet (UV) radiation

Data on levels of ambient UV radiation were provided by the Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) and measured as measured in Standard Erythemal Doses (available at www.arpansa.gov.au/our-services/monitoring/ultraviolet-radiation-monitoring/ultraviolet-radiation-dose)

Statistical consideration and analysis

The study was powered to detect a difference in serum 25(OH)D_3 levels between the two groups at baseline. Using the results of the study by Harries and colleagues[21] that showed lower levels of 25(OH)D_3 in CD patients, the study was powered to detect an 8% difference in the 25(OH)D_3 level between the two groups with an α of 0.05 and β of 0.80. On this basis, we determined a sample size of 27 per group was required. Longitudinal analysis of the 6-month data was exploratory and the study was not powered for this analysis.

Summary statistics include counts and percentages for categorical variables and mean [standard deviation (SD)] or median [interquartile range (IQR)] for continuous variables, depending on whether the distribution was normal or non-normal, respectively. Group comparison of continuous variables were assessed using Student's unpaired *t* test for parametric data, or Mann-Whitney *U* and Kruskall-Wallis testing for non-parametric data. Categorical variables were compared using Pearson chi-square test or Fisher's exact test.

A score for vitamin D obtained from sunlight exposure was calculated using a modified method described by Cargill and colleagues.[20] The reported time spent outdoors between 10 am and 3 pm during an average week was combined with a weighted UV multiplier based on the month the data were collected. Finally, a multiplier was applied based on the reported clothing coverage to estimate body surface area exposed (see Supplementary Data Content 2 for further details).

A score for vitamin D intake was generated from dietary sources using the dietary questionnaires combined with the reported vitamin D content of foods as described by the Nutritional Tables (NUTTAB) 2010 from Food Standards Australia New Zealand (available from http://www.foodstandards.gov.au).

The sunlight and dietary scores were then combined using the following process. Separate linear regression models assessing the change in measured 25(OH)D concentration in participants who remained with inactive disease throughout the study were computed using the sunlight or dietary scores as independent variables in their respective model. The calculated beta coefficients from each model (diet or sunlight) were then used as weights to calculate a combined score of external vitamin D determinants. This combined score was used as a controlling variable in the main analysis to ensure the difference in 25(OH)D_3 and its metabolites were related to underlying disease activity rather than vitamin D intake or sunlight exposure.

A linear regression model was used in the cross-sectional analysis to test the association between vitamin D metabolites and disease activity and in the longitudinal analysis to assess change in vitamin D metabolites in respect to changing disease activity. Outputs from the analysis included the marginal mean [95% confidence interval (CI)], marginal mean difference (95% CI) and the *p* value.

All statistical analyses were completed on IBM® SPSS® Statistics version 25 (IBM Corp, Armonk, NY, USA). A p value of <0.05 was considered statistically significant.

Ethical considerations

The study protocol was approved by the St. Vincent's Hospital Human Research Ethics committee (LNR/17/SVH/26). Written informed consent was obtained from all participants.

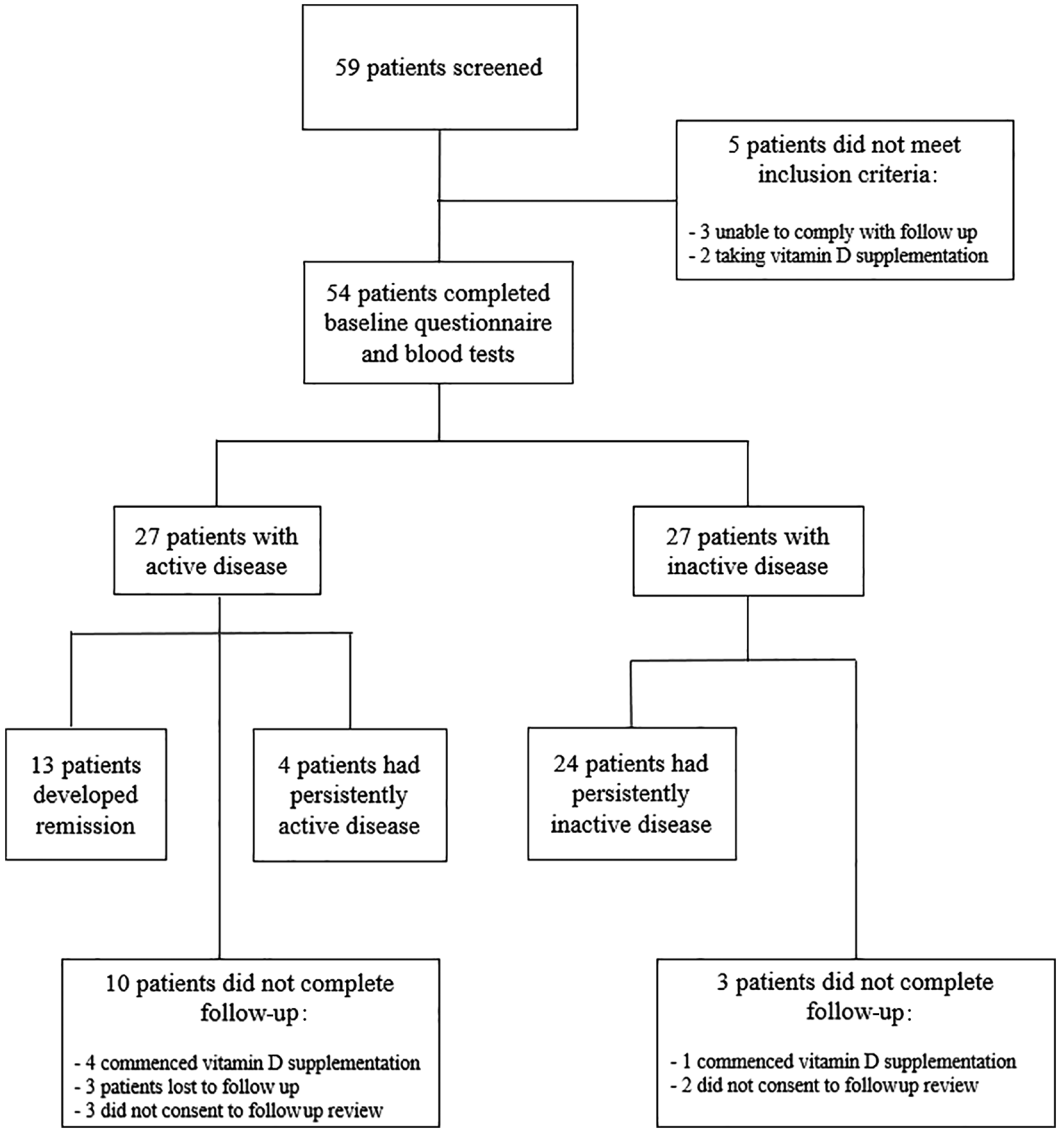

Figure 2. Patient enrolment and follow up.

Results

Patient characteristics

Fifty-nine consecutive patients with CD meeting the inclusion criteria were identified (Figure 2). Five patients were excluded either due to the inability to comply with required follow up or current vitamin D supplementation that was not identified on screening. A total of 54 participants were included in the final analysis; 27 with active disease and 27 with inactive disease. Thirty-one patients (54%) were male and the mean age was 37 years (Table 1). All patients had colonic involvement, with 24 (42%) having isolated colonic disease (Montreal Classification L2) and the remaining 30 (58%) having ileocolonic disease (Montreal Classification L3). Ten patients (17%) had a history of perianal CD, though no patients had active perianal disease. As per the inclusion criteria, none of the patients were receiving vitamin D supplementation or corticosteroids for 4 weeks prior to enrolment.

Of the 27 patients with active disease, 24 (89%) were included based on a recent ileocolonoscopy showing active disease, with the remainder having an elevated faecal calprotectin.

There were no significant differences in age, disease phenotype, previous intestinal resections,

Table 1. Baseline characteristics: comparison of variables between participants with active disease and inactive disease.

	Active disease *n* = 27	Inactive disease *n* = 27	*p* value
Sex			
Male, *n* (%)	17 (63)	14 (52)	0.40
Age, mean (SD), years	39.3 (14.3)	36.4 (9.0)	0.39
Age at diagnosis, *n* (%) (Montreal classification)			0.14
A1	3 (11)	1 (4)	
A2	18 (67)	24 (89)	
A3	6 (22)	2 (7)	
Disease location, *n* (%) (Montreal classification)			0.58
L2	11 (41)	13 (48)	
L3	16 (59)	14 (52)	
History of perianal disease, *n* (%)	3 (11)	7 (26)	0.16
Smoking status, *n* (%)			0.89
Never	14 (52)	15 (56)	
Current	3 (11)	2 (7)	
Exsmoker	10 (37)	10 (37)	
Immunomodulator, *n* (%)	9 (33)	17 (63)	0.03
Biological therapy, *n* (%)			<0.001
Tumour necrosis factor	7 (26)	22 (82)	
Vedolizumab	3 (11)	1 (4)	
Albumin, mean (SD), g/l	41.0 (4.6)	46.7 (3.1)	<0.001
C-reactive protein, median (IQR), mg/l	5.3 (19.8)	0.7 (2.4)	<0.001
BMI, mean (SD), kg/m^2	24.7 (7.6)	24.0 (4.4)	0.68
Skin tone, *n* (%)			0.07
Very fair	4 (15)	5 (19)	
Fair	9 (33)	14 (52)	
Light olive	13 (48)	6 (22)	
Brown	1 (4)	2 (7)	
External determinants of vitamin D score, mean (SD)			
Diet	336 (201)	245 (125)	0.59
Sunlight	63 (78)	39 (52)	0.18
Combined	127 (48)	83 (48)	0.04

BMI, body mass index; IQR, interquartile range; SD, standard deviation.

body mass index (BMI) or smoking status among those with inactive compared with active disease (Table 1). Patients with inactive disease were more likely to be on an immunomodulator ($p = 0.003$) or biological therapy ($p < 0.001$) and have higher mean serum albumin concentration ($p < 0.001$).

Patients with active disease had higher dietary intake of vitamin D as well as sunlight exposure, collectively represented as external determinants of 25(OH)D levels (Table 1).

Levels of vitamin D metabolites: baseline

Based on the concentration of $25(OH)D_3$, only seven (13%) patients were in the vitamin-D-sufficient range [$25(OH)D_3 \geqslant 75$ nmol/l] as defined by the US Endocrine Society.[6] A total of 29 (51%) had vitamin D insufficiency (50–74 nmol/l) and 18 (32%) were in the deficient range (<50 nmol/l; Table 2). There were no significant differences in $25(OH)D_3$ or $1,25(OH)_2D_3$ levels between those with active or inactive disease. However, participants with active disease had a significantly lower $24,25(OH)_2D_3$, and therefore had a higher $25(OH)D_3$:$24,25(OH)_2D_3$ ratio (Table 2). In the active disease group, there was a trend to a higher VDBP and there was a lower calculated bioavailable $25(OH)D_3$ (Table 2).

Using linear regression modelling to control for external determinants of vitamin D, $24,25(OH)_2D_3$ and bioavailable $25(OH)D_3$ levels were significantly lower among those with active disease and the ratio of $25(OH)D_3$:$24,25(OH)_2D_3$ was significantly higher (Table 3). There were no significant differences in serum $25(OH)D_3$, $1,25(OH)_2D_3$, VDBP or free $25(OH)D_3$ levels between patients with active and inactive disease. Furthermore, there was no significant correlation between dietary, sunlight or combined vitamin D exposure with $25(OH)D_3$ or its metabolites.

Analysis at 6-month follow up

Follow-up analysis, including repeated sunlight and dietary questionnaires and measurements of vitamin D metabolites, was completed for 41 patients. At the time of follow up, no patients were taking corticosteroids. Of the 17 patients with active disease at enrolment that completed follow up, 13 (76%) developed remission during the follow-up period. Four patients had persistently active disease and no patients with inactive disease at the start of the study period developed a flare of disease (Figure 2).

In patients with active disease who developed clinical remission during the follow-up period, there was a significant increase in the mean $25(OH)D_3$ from baseline to 6 months, such that the mean $25(OH)D_3$ returned to sufficient levels as defined by the US Endocrine Society [$25(OH)D_3 \geqslant 75$ nmol/l]. After controlling for the change in external determinants of vitamin D, levels of $25(OH)D_3$ increased significantly more in patients whose disease activity improved as compared with patients who had persistently active, or inactive, disease throughout the study period (Table 4). The increase in $25(OH)D_3$ was accompanied by a significant increase in the mean $24,25(OH)_2D_3$ concentration to a level that was similar to levels found in patients who remained in remission throughout the study ($p = 0.96$; data not shown).

Discussion

To date, there has been debate on whether vitamin D deficiency is causally linked to the development, activity and complications of IBD. Many of the association studies reporting this link do not adequately control for potential confounders such as corticosteroid use, vitamin D intake and sunlight exposure. This is the first study, to our knowledge, to prospectively examine the metabolism of vitamin D using the gold standard LC/MS/MS in a cohort of patients with CD not on corticosteroids with similar disease distribution and also carefully controlling for sunlight exposure and vitamin D intake to determine the independent effect of intestinal inflammation on circulating vitamin D metabolite levels.

Contrary to existing literature, we did not find reduced $25(OH)D_3$ levels in patients with active CD despite an appropriate sample size to detect this difference. There are several possible explanations for this. First, the location of the study, Sydney, Australia (latitude 33° 87' S), has a much higher UV Index compared with that of many North American and European cities from where the existing literature originates. For example, the average summer UV Index in Sydney is 9–11, whereas in Denmark, where one of the earlier association studies was performed, the average summer UV Index is 5–6.[1,22,23] Thus, the Sydney population is likely to be more resistant to

Table 2. Baseline vitamin D metabolite levels.

	Active disease *n* = 27 (%)	Inactive disease *n* = 27 (%)	*p* value
$25(OH)D_3$ (nmol/l)			0.51
0–49	11 (41)	7 (26)	
50–74	13 (49)	16 (59)	
75+	3 (11)	4 (15)	
$25(OH)D_3$, mean (SD), nmol/l	59.2 (26.3)	60.0 (22.0)	0.91
$24,25(OH)_2D_3$, mean (SD), µmol/l	3.9 (2.3)	6.0 (2.9)	0.007
Ratio of $25(OH)D_3$: $24,25(OH)_2D_3$ mean (SD)	17.3 (7.9)	11.1 (3.9)	0.001
$1,25(OH)_2D_3$, mean (SD), pmol/l	114.0 (56.0)	117.5 (39.6)	0.80
VDBP, mean (SD), µmol/l	5.6 (1.3)	5.0 (0.9)	0.07
Bioavailable $25(OH)D_3$ mean (SD), nmol/l	4.7 (2.5)	6.0 (1.9)	0.05
Free $25(OH)D_3$, mean (SD), pmol/l	14.3 (5.8)	15.4 (4.8)	0.41

$25(OH)D_3$, 25-hydroxy vitamin D_3; SD, standard deviation.

Table 3. Results from the multiple linear regression model testing of baseline vitamin D metabolites across the active and inactive disease groups, controlling for external sources of vitamin D.

Variable	Active disease marginal mean (95% CI)	Inactive marginal mean (95% CI)	Mean difference (95% CI)	*p* value
$25(OH)D_3$ (nmol/l)	57.9 (48.7–67.0)	61.3 (52.2–70.6)	−3.51 (−16.7 to 9.71)	0.60
$24,25(OH)_2D_3$ (µmol/l)	3.9 (2.9–4.9)	6.1 (5.1–7.1)	−2.3 (−3.7 to −0.8)	0.002
$25(OH)D_3$:$24,25(OH)_2D_3$	17.2 (14.9–19.6)	11.1 (8.7–13.5)	6.1 (2.7–9.6)	<0.001
$1,25(OH)_2D_3$ (pmol/l)	114.6 (93.7–135.5)	117.0 (98.8–135.2)	−2.4 (−30.7 to 25.9)	0.87
VDBP (µmol/l)	5.5 (5.1–5.9)	5.0 (4.6–5.5)	0.5 (−0.1 to 1.1)	0.13
Free $25(OH)D_3$ (pmol/l)	14.0 (12.0–16.1)	15.7 (13.7–17.8)	1.7 (−4.6 to 1.2)	0.26
Bioavailable $25(OH)D_3$ (nmol/l)	4.7 (3.9–5.6)	6.1 (5.2–6.9)	−1.3 (−2.5 to −0.08)	0.04

$25(OH)D_3$, 25-hydroxy vitamin D_3; CI, confidence interval; VDBP, vitamin-D-binding protein.

developing vitamin D deficiency, even in the face of active inflammation. Second, this study excluded patients on corticosteroids at baseline which is known to reduce $25(OH)D_3$ levels and has been a confounding factor in the published literature.[8] For example, in the previously mentioned cross-sectional Danish study, 25(OH)D levels were inversely correlated with disease activity as measured by CDAI and CRP. While the authors examined the effect of smoking and BMI as potential confounders, the use of corticosteroids was not discussed.[1]

Table 4. Results of a multiple linear regression model of changes in vitamin D metabolites at 6-months' follow up.

Variable	Group	Mean difference between baseline and 6 months (95% CI)	Marginal mean difference between groups of patients (95% CI)	*p* value
$25(OH)D_3$ (nmol/l)[1]	Active to remission	32.3 (16.6–47.90)	20.71 (1.4–40.0)	0.03
	Persistently active	6.9 (–20.7 to 34.6)	–4.6 (–34.7 to 25.4)	0.76
	Persistently inactive	11.6 (0.2–22.8)	Reference	Reference
$24,25(OH)_2D_3$ (µmol/l)[2]	Active to remission	2.1 (0.8–3.5)	2.3 (0.6–4.1)	0.008
	Persistently active	1.0 (–1.4 to 3.3)	1.2 (–1.5 to 3.8)	0.38
	Persistently inactive	–0.2 (–1.2 to 0.8)	Reference	Reference
$25(OH)D_3$:$24,25(OH)_2D_3$[3]	Active to remission	4.4 (0.2–8.6)	–0.5 (–5.9 to 4.9)	0.85
	Persistently active	–0.3 (–7.3 to 6.7)	–5.2 (–13.0 to 2.5)	0.18
	Persistently inactive	4.9 (1.9–7.9)	Reference	Reference
VDBP (µmol/l)[4]	Active to remission	0.24 (–0.13 to 0.62)	0.24 (–0.23 to 0.71)	0.31
	Persistently active	–0.27 (–0.94 to 0.41)	–0.27 (–1.02 to 0.46)	0.46
	Persistently inactive	0.004 (–0.27 to 0.28)	Reference	Reference
Free $25(OH)D_3$ (pmol/l)[5]	Active to remission	6.5 (2.8–10.19)	3.3 (–1.2 to 7.9)	0.15
	Persistently active	1.6 (–4.9 to 8.1)	–1.5 (–8.7 to 5.6)	0.67
	Remission	3.2 (0.5–5.8)	Reference	Reference
Bioavailable $25(OH)D_3$ (nmol/l)[6]	Active to remission	1.7 (–0.1 to 3.4)	0.8 (–1.4 to 3.0)	0.46
	Persistently active	–0.1 (–3.2 to 3.0)	–0.9 (–4.4 to 2.5)	0.59
	Persistently inactive	0.8 (–4.4 to 2.1)	Reference	Reference

The change in vitamin D metabolite levels over the 6 months from baseline to follow up is shown for participants that remained in active disease, inactive disease or were originally in active disease but achieved remission. Data were analysed using a multiple linear regression model with the following adjustments:
[1]Adjusted for baseline $25(OH)D_3$ and change in external determinants of vitamin D.
[2]Adjusted for baseline $24,25(OH)_2D_3$.
[3]Adjusted for baseline $25(OH)D_3$:$24,25(OH)_2D_3$.
[4]Adjusted for baseline VDBP.
[5]Adjusted for baseline free $25(OH)D_3$ and change in external determinants of vitamin D.
[6]Adjusted for baseline bioavailable $25(OH)D_3$ and change in external determinants of vitamin D.
$25(OH)D_3$, 25-hydroxy vitamin D_3; CI, confidence interval; VDBP, vitamin-D-binding protein.

When assessing the metabolism of vitamin D, levels of $24,25(OH)_2D_3$ were significantly lower in participants with active disease. This was contrary to our original hypothesis which proposed that active inflammation would lead to catabolism of $25(OH)D_3$, resulting in increased concentrations of $24,25(OH)_2D_3$. One explanation could be that in the setting of active inflammation there is relative $25(OH)D_3$ deficiency which leads to reduced catabolism to maintain levels of circulating $25(OH)D_3$, and the metabolically active $1,25(OH)_2D_3$, which were not different between the groups in our study. When disease activity improved, levels of $24,25(OH)_2D_3$ returned to levels that were similar to those seen in patients who remained with inactive disease throughout the study period. In one other study, $24,25(OH)_2D_3$ metabolites were measured using a radioimmunoassay in well-nourished and undernourished adult CD patients ($n=40$) compared

with UC ($n=20$) and healthy controls ($n=9$).[3] In that study, $25(OH)D_3$ but not $24,25(OH)_2D_3$ or $1,25(OH)_2D_3$ levels were lower in those with active CD. The disparate results from that study compared with the current study could have been due to methodological differences. For example, in this previous study, there was a large variability in the $24,25(OH)_2D_3$ measurements likely indicating the limitations of the radioimmunoassay used. In addition, disease activity was defined using a simple clinical index without supporting inflammatory markers. Symptoms of CD correlate poorly with objective markers of inflammation and therefore in our study, we included an objective marker of inflammation as part of the active disease criteria with 89% of participants having had a recent ileocolonoscopy showing active disease and others had elevation of faecal calprotectin. These factors may explain the differing results found in our study.

Participants who achieved clinical remission during the study period experienced a spontaneous and significant increase in $25(OH)D_3$ levels to a level considered in the sufficient range, even after controlling for baseline levels, sunlight and dietary intake. This is consistent with a study of 37 patients with CD, where an early increase in serum $25(OH)D_3$ was observed in those responding to tumour necrosis factor (TNF) inhibitors. This is an important observation, as it supports the concept of $25(OH)D_3$ being a negative acute-phase reactant rather than a driver of disease activity. Indeed, studies have suggested that vitamin D sufficiency improves the likelihood of achieving remission in CD with TNF inhibitors.[24] While this may be possible, it is also possible that those patients with higher $25(OH)D_3$ levels have less inflammatory burden and thus, are more likely to respond to biological therapy and by treating the underlying inflammation, $25(OH)D_3$ concentrations are able to spontaneously recover. Another potential mechanism for the improved $25(OH)D_3$ concentrations is that in the setting of intestinal inflammation, there may be partial vitamin D malabsorption which is corrected with improvement of underlying inflammation. Certainly, in our cohort of patients with active CD, 59% had ileal inflammation (Montreal L3) and resolution of this inflammation may have contributed to the findings seen in this study.

There was a trend for increased VDBP levels and lower calculated bioavailable vitamin D in the setting of active disease compared with inactive disease. This is consistent with our previous reports of elevated VDBP levels predicting subsequent disease relapse in patients with CD, as well as seen in murine models of colitis.[14] One potential mechanism for this association is that the presence of lower bioavailable vitamin D may impair innate and adaptive immune responses as well as barrier function, impeding the resolution of inflammation. In contrast to our findings, VDBP levels fall in the setting of critical illness, not specifically related to gastrointestinal disease.[25] This difference in observations is that the rise in VDBP concentration in the setting of CD may be uniquely related to a process that occurs in intestinal inflammation. Furthermore, in this study, we estimated bioavailable vitamin D through validated calculations; however, future studies should utilize techniques to directly measure circulating levels.

Several limitations warrant mention. First, the sample size was small, but we reached the predetermined sample size for the baseline cross-sectional analysis. It is possible that with a larger cohort, differences in $25(OH)D_3$ levels may have been observed; however, this is unlikely given the small, clinically insignificant difference seen between groups seen (0.08 mmol/l) and p value obtained ($p=0.91$).

None of the participants with inactive disease relapsed during the follow-up period. This is not unexpected and consistent with recent literature showing that $<10\%$ of patients develop disease flare over a 1-year period when in stable remission on immunosuppression, especially with anti-TNF treatment.[26] Therefore, we were unable to demonstrate changing concentrations of 25(OH)D and its metabolites with disease flare. With such infrequent relapses, a very large study population would have been required to adequately power a longitudinal study. Despite this, our exploratory analysis did reveal significant changes in several parameters. A longer follow up or a larger cohort may further delineate the impact of disease relapse on vitamin D metabolites.

Measuring external vitamin D sources using retrospective questionnaire data has limitations with UV dosimeters remaining the gold standard for measuring exposure to UV-B radiation. However, previous studies have shown that measurements from UV dosimeters explained only 8.3% of the

variance in 25(OH)D levels.[27] The questionnaires used in this study have been validated against UV-dosimeter data and thus, are felt to be a reasonable estimate of sunlight exposure for the purpose of this study.

Conclusion

In the setting of active CD and no concurrent corticosteroids, $24,25(OH)_2D$ but not 25(OH)D levels were reduced. This may be related to an innate homeostatic mechanism to maintain circulating 25(OH)D and 1,25(OH)D levels in the setting of relative deficiency. Importantly, levels of 25(OH)D spontaneously rose with the treatment of underlying inflammation, suggesting that aggressive supplementation may not be necessary. The significance of $24,25(OH)_2D$ as a potential marker of vitamin D status and CD activity requires further exploration.

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Conflict of interest statement

The author(s) declare that there is no conflict of interest.

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Supplemental material

Supplemental material for this article is available online.

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