

Vitamin D as a Novel Therapy in Inflammatory Bowel Disease – New Hope or False Dawn?

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Abstract

There is increasing scientific interest in the field of vitamin D research, moving the focus beyond bone health to other disease processes. Low circulating vitamin D levels have been reported as a risk factor for several pathophysiologically divergent diseases including cancers, diabetes, cardiovascular diseases, multiple sclerosis and inflammatory diseases including rheumatoid arthritis and inflammatory bowel disease (IBD). But, therein, remains the challenge - can any single nutrient contribute to multiple complex disease mechanisms and, ultimately, have therapeutic potential?

The aim of this review is to critically evaluate several strands of scientific evidence surrounding vitamin D and inflammation, primarily focussing on IBD. Epidemiological studies suggest an increased incidence of IBD and rheumatoid arthritis in countries of more Northern latitudes, mirroring sunlight patterns. A considerable body of evidence supports the anti-inflammatory effects of vitamin D, at least in animal models of IBD. While it is accepted that suboptimal vitamin D status is common in IBD, some studies suggest that this associates with more severe disease. With regard to treatment, the data are only beginning to emerge from randomised controlled trials (RCTs) to suggest that people with IBD may remain in remission longer when treated with oral vitamin D. In conclusion, several strands of evidence suggest that vitamin D may modify the immune response in IBD. There is a continued need for large well-designed clinical trials and mechanistic studies to determine if, and how, this emerging promise translates into tangible clinical benefits for people with chronic debilitating diseases such as IBD.

Vitamin D and Immunomodulation: the Inflammatory Bowel Disease Perspective

There is increasing scientific interest in the field of vitamin D research, moving the focus beyond traditional known roles in bone health to other disease processes. Maintaining adequate serum vitamin D levels has been associated with a reduced risk of several pathophysiologically divergent diseases including cancers, diabetes, cardiovascular diseases, multiple sclerosis and inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease (IBD) ⁽¹⁾. But, therein, is the challenge - can any single nutrient contribute to multiple complex disease mechanisms and, ultimately, have potential to treat these diseases? Across a number of the autoimmune diseases including rheumatoid arthritis, multiple sclerosis, asthma, systemic lupus erythematosus (SLE) and IBD, studies suggest that vitamin D status may be associated with initiation, progression or severity of these diseases ^(1; 2; 3; 4; 5; 6).

In IBD, there is an established role for vitamin D in the prevention and treatment of osteoporosis, which is a known complication of this disease ^(7; 8; 9). Indeed, there are clinical management guidelines for bone health ⁽¹⁰⁾, for example, that recommend vitamin D and calcium supplementation for IBD patients undergoing treatment with corticosteroids. There is new interest, however, in vitamin D in IBD 'beyond bone' as a treatment for the core inflammatory disease. Growing evidence from epidemiological studies, animal models, cross-sectional studies and some intervention studies appear to support such a role ^(11; 12; 13; 14; 15). The aim of this review is to critically evaluate several strands of scientific evidence surrounding vitamin D and inflammation in autoimmune diseases, primarily focussing on IBD and to understand how this translates to disease management.

Inflammatory Bowel Disease encompasses two conditions, Crohn's disease (CD) and a related condition, ulcerative colitis (UC). Crohn's disease is a lifelong chronic relapsing and remitting inflammatory condition affecting any part of the gastrointestinal tract. Symptoms include diarrhoea, abdominal pain, fever and fatigue. The disease is named after Dr. Burrill Crohn, who in 1932, along with his colleagues, published a landmark paper describing a condition now known as Crohn's disease ⁽¹⁶⁾. Ulcerative colitis is a similar entity, but as the name suggests inflammation is confined to the colon (colitis); therefore, unlike CD surgical removal of the colon can be curative in UC. IBD is associated with increased morbidity, hospitalisations, surgery, medical and nutritional complications and high health care costs.

For people with this condition it can be debilitating and result in poor quality of life and life-long ill health.

CD cannot yet be cured, and therefore disease management focuses on pharmacological interventions that control symptoms and maintain remission ⁽¹⁷⁾ with many patients requiring surgery at some stage during their disease course ⁽¹⁸⁾. Several nutritional approaches aimed at maintaining remission in CD have been investigated, including fish oils, probiotics and enteral nutrition, although none have translated to effective mainstream management for adult CD ^(6; 19; 20). In recent years, vitamin D has emerged as a candidate of interest as an adjunctive treatment in CD. In this context, most of the published human studies of vitamin D as adjunctive treatment of IBD have been conducted in CD, and consequently CD constitutes much of the focus of much of this review paper.

Vitamin D Deficiency is Common in CD – Cause or Effect?

Vitamin D deficiency is common in IBD. Prevalence of deficiency may range from 35-100% in CD ⁽²¹⁾, when deficiency is defined as circulating 25-hydroxyvitamin D [25(OH)D] less than 50 nmol/L ⁽²²⁾ and applied across a range of published studies. Studies reporting suboptimal vitamin D status in CD have considerable methodological variation, including differences in the definitions of deficiency, sample size, geographic region, season, characteristics of the study cohorts and in the vitamin D assay used. General debate around what level of 25(OH)D is considered deficient or insufficient further complicates the issue ⁽²³⁾, and whether a higher threshold for deficiency should be applied in disease states such as CD. Despite differences in these studies, it is clear that suboptimal vitamin D status is common in CD. Indeed, this is not surprising considering the high background level of vitamin D deficiency in healthy individuals and the added risk of deficiency conferred by a chronic, inflammatory gastrointestinal disease.

In CD, both generic and disease-specific factors contribute to circulating levels of 25(OH)D attained. These include sunlight exposure, skin pigmentation, indoor sedentary lifestyles, obesity, cigarette smoking, and dietary and supplementary vitamin D intake. For those with CD, there are additional risk factors for deficiency such as malabsorption, intestinal inflammation, intestinal resection, corticosteroid usage and poor dietary intake. High prevalence of suboptimal vitamin D status is not disputed in IBD, but it is not clear whether this has the capacity to influence CD initiation and severity or is merely a consequence of the disease.

Vitamin D Status as a Risk Factor for Developing IBD

The exact cause of IBD remains unknown; however, the disease is thought to result from a complex interaction between immunological, genetic and environmental factors. Vitamin D status is one such postulated environmental risk factor^(24; 25; 26). Epidemiological studies show a geographic variation in the incidence of IBD, with a higher incidence in countries of more northern latitudes, mirroring sunlight patterns and likely to reflect vitamin D levels. Studies in the US^(25; 27) and Europe have linked latitude to risk of both CD and UC^(28; 29; 30).

Two separate published analyses, based on data from women who participated in the Nurses' Health studies in the US, support this association between higher latitude and higher incidence of IBD^(25; 27). Khalili *et al* reported a significant increase in incidence of both CD and UC according to more northerly latitude of residence⁽²⁵⁾. The authors further analysed latitude of residence across a number of time points and found the strongest association at age 30 years. For example, compared with women living at northern latitudes at age 30, the multivariate-adjusted HR for women living in southern latitudes was 0.48 (CI 0.30 to 0.77) for CD and 0.62 (CI 0.42 to 0.90) for UC⁽²⁵⁾. Since IBD is likely to develop over a considerable period of time, understanding the role of risk factors, such as latitude of residence or vitamin D status, at different age points may be useful. In a further study, using an estimate of predicted vitamin D status, Ananthakrishnan *et al*⁽²⁷⁾ showed that higher predicted 25(OH)D was significantly associated with a reduced risk for incident CD [hazard ratio (HR) 0.54, P_{trend} 0.02; CI 0.30-0.99] but not for ulcerative colitis [HR 0.65 (P_{trend} 0.17; CI 0.34-1.25)].

To date, investigations into vitamin D and risk of IBD have not directly measured circulating 25(OH)D levels, but instead have used surrogate markers such as latitude of residence or an index for predicted vitamin D⁽³¹⁾ status. Residing at southern latitudes would be expected to increase an individual's access to the UVB rays responsible for cutaneous vitamin D synthesis. In general, it is thought that for people living above 40⁰N, for example north of Rome or Chicago, sunlight alone is unlikely to maintain adequate vitamin D status all year around, and thus requires a reliance on dietary sources and body stores of vitamin D⁽³²⁾.

Geographic location may, of course, reflect disease risk factors other than 25(OH)D levels, for instance, genetic or environmental influencers. While newer theories suggest that other components of sunlight (e.g. ultraviolet-A non-vitamin D making rays) might also have health effects, independent of vitamin D⁽³³⁾. The association with disease incidence and

latitude is not unique to IBD, there is supporting parallel evidence of a north-south gradient in the incidence of other autoimmune and gastrointestinal diseases including rheumatoid arthritis ⁽³⁴⁾ and colorectal cancer ^(35; 36; 37). For colorectal cancer, geographic region ⁽³⁷⁾, predicted ⁽³⁸⁾ and measured pre-diagnostic circulating 25(OH)D have been linked with disease risk ⁽³⁶⁾.

Vitamin D Status and Associations with Disease Severity in CD

The relationship between circulating 25(OH)D concentrations and disease activity in CD has been explored in several cross-sectional studies, yet the findings remain inconsistent. Some studies suggest that low 25(OH)D is associated with more active disease, while others do not support this premise, based on studies collectively comprising approximately 800 CD participants (table 1). Understanding this association is complicated by the different parameters applied by studies to capture disease activity, which typically include:

- a) clinical activity scores, namely the Crohn's disease activity index (CDAI), currently the gold standard ⁽³⁹⁾, or the Harvey Bradshaw Index ⁽⁴⁰⁾
- b) C-Reactive Protein (CRP) as a marker of systematic inflammation
- c) faecal calprotectin ⁽⁴¹⁾ as a marker of intestinal inflammation.

For systematic inflammation, as determined by CRP, current studies broadly agree and show a lack of association with 25(OH)D levels. The two studies to date that have investigated intestinal inflammation using faecal calprotectin show a significant inverse association between 25(OH)D and disease activity ^(42; 43). Analysis according to clinical disease activity scores (CDAI or HBI), however, lack agreement (table 1) in cross-sectional studies. Importantly, some study populations included only patients in disease remission ⁽²¹⁾, while others comprised patients with active disease and those in remission ⁽⁴²⁾. In addition to disease activity, other authors have indicated an association between low 25(OH)D and increased need for surgery and hospitalisations for CD ⁽⁴⁴⁾ which are considered to reflect more severe disease. An association with low vitamin D levels and loss of response to immunomodulatory treatments has also been reported ⁽⁴⁵⁾.

Collectively, observational studies provide evidence of the co-existence of lower vitamin D levels and more active disease, nevertheless, the issue of 'cause and effect' cannot be answered by these studies. The question remains as to whether interventions to raise 25(OH)D levels by vitamin D supplementation in these patients would modify disease

activity or alter disease progression? This question is now beginning to be addressed by intervention studies.

Vitamin D as Therapy for CD – Evidence from Intervention Studies

Currently there are few published randomised controlled trials (RCTs) that have investigated vitamin D as a therapeutic agent to prevent relapse or induce remission in CD (Table 2). The key randomised controlled study published by Jorgensen and colleagues in 2010⁽¹⁴⁾ showed a non-significant reduction in relapse rates in CD patients treated with 1200 IU vitamin D₃ daily for 12 months. Patients (n=98) were in clinical remission at enrolment, and at 12 months the relapse rates were 12% and 29% in the vitamin D treated and placebo groups respectively (p=0.06). An open-label study from 2009, designed to examine the effects of vitamin D on bone markers in CD (n=37)⁽⁴⁶⁾ also captured effects on disease activity. Patients were treated with either active vitamin D or 1000 IU D₃ daily for 12 months; the authors reported a significant short term reduction in disease activity (CDAI and CRP) with a more pronounced effect noted for the active form of vitamin D⁽⁴⁶⁾.

More recently, Yang *et al*⁽⁴⁷⁾ applied a protocol designed to focus on achieving circulating 25(OH)D levels of 100 nmol /L rather than on administering a predefined fixed daily dose of vitamin D. In this small study (n=18), patients with mild to moderately active CD were supplemented with oral vitamin D₃; this dose was initiated at 1000 IU daily and escalated up to a maximum of 5000 IU, to achieve the target circulating 25(OH)D of 100 nmol/L. The key finding was a significant reduction in CDAI scores at 6 months. Focussing on achieving a target 25(OH)D level is interesting, since some authors suggests that levels of 75nmol/L or higher may be required to observe changes in non-skeletal outcomes^(48; 49). Preliminary findings from a randomised controlled study from our group⁽⁵⁰⁾, showed that CD patients who achieved 25(OH)D levels ≥ 75 nmol/L has significantly lower serum CRP than those with levels below this threshold – this was in response to a 3 month treatment with 2000 IU/day vitamin D₃ in patients in remission.

Taken together, results from intervention studies suggest that vitamin D supplementation may reduce markers of disease activity in CD. Although at this point, there is insufficient evidence to support vitamin D as an anti-inflammatory therapy. There is a continued need for high quality RCTs for vitamin D therapy IBD. In essence, vitamin D clinical trials should be well-designed and employ outcome measures compatible with other therapeutic studies. Ideally, evidence of intestinal mucosal healing is a desirable outcome measure in response to vitamin

D supplementation; although this requires an invasive endoscopic examination which is logistically challenging for many nutrition studies. As an alternative, measurement of faecal calprotectin as a surrogate marker of intestinal inflammation may be useful ⁽⁴¹⁾. Furthermore, there is interest in other endpoint measures such as patient-reported outcomes that may be applicable to clinical trials ⁽⁵¹⁾. Other aspects to consider include factors predicting response and failure to vitamin D treatments. For example, is response determined by CD characteristics, phenotype or genotype or influenced by vitamin D genotype? In line with this, a RCT in tuberculosis identified a better response to adjunctive vitamin D therapy in participants with the tt genotype of the TaqI vitamin D receptor polymorphism ⁽⁵²⁾.

Vitamin D as Therapy – Is There a Therapeutic Zone for 25(OH)D Levels?

In intervention studies, the level of circulating 25(OH)D achieved as well as the dose of supplemental vitamin D are likely to be important factors in understanding the therapeutic response. While circulating 25(OH)D >50 nmol/L has been deemed adequate for bone health ⁽²²⁾, immunomodulatory effects may require higher levels. Indeed, concentrations of >75, or indeed 90-100 nmol/L 25(OH)D have been proposed for multiple health outcomes ⁽⁴⁸⁾, though others argue the case for even higher levels of 100-200 nmol/L ⁽²³⁾. In the context of CD, a target 25(OH)D concentration deemed optimal for anti-inflammatory or therapeutic effects, if any, is not known. In the few RCTs of vitamin D in CD conducted to date (table 2) levels of 75-100 nmol/L 25(OH)D have been achieved. Jorgensen *et al*, for instance, achieved a mean 25(OH)D just below 100 nmol/L (mean 96 nmol/L, sd 27) in response to 1200 IU/day vitamin D treatment; this was accompanied by a non-significant reduction in CD relapse rates at 1 year ⁽¹⁴⁾. Preliminary findings from our pilot intervention study, showed that 2000 IU/day vitamin D raised levels from 69 to 92 nmol/L at 3 months, which was accompanied by a modest reduction in CRP but only in participants who achieved serum 25(OH)D >75nmol/L ⁽⁵⁰⁾. Yang *et al* ⁽⁴⁷⁾ specifically designed their study to achieve a target circulating 25(OH)D of 100 nmol/L, and achieving this target level was associated with short term improvements in disease activity.

Vitamin D therapy poses additional challenges compared with other therapeutic trials. In pharmaceutical trials, the placebo group is typically unexposed to the therapeutic intervention, whereas in vitamin D clinical trials the placebo group have an existing background level of vitamin D, which may vary in participants. Both Jorgensen *et al* and Raftery *et al* ⁽⁵⁰⁾ reported a good baseline 25(OH)D level at study enrolment, (69 nmol/L for

both studies). It is plausible that supplementation in a vitamin D deficient CD group would be of greater benefit compared to a replete group. It is, as yet, unclear how baseline vitamin D status, levels attained post-treatment and the magnitude of change from baseline influence treatment response.

Thus far, modest changes in disease activity in CD have been observed in intervention studies that achieved vitamin D levels in the region of 75-100nmol/L. On balance, there is as yet insufficient evidence to make a judgment about a therapeutic or immunomodulatory zone for 25(OH)D superficially for CD. Clinically, our group currently apply a cut-off of 25(OH)D < 50 nmol/L for vitamin D deficiency. According to the US Institute of Medicine report, ⁽²²⁾ levels higher than 50 nmol/L are considered to cover the requirements of at least 97.5% of the healthy population. The US Endocrine Society guidelines ⁽²³⁾, however, define vitamin D deficiency as 25(OH)D <50nmol/L, but consider <75 nmol/L as vitamin D insufficiency ⁽²³⁾. Although there remains debate about disease-specific and health outcome-specific levels. In the meantime, it seems prudent that, at a minimum, clinical management should aim to prevent vitamin D deficiency in CD.

Vitamin D as Therapy in CD – Evidence of Underlying Mechanisms

During the 1980s vitamin D receptors (VDRs) were identified on cells of the immune system ^(53; 54) sparking interest in immunomodulating effects of vitamin D. For IBD, there is a compelling body of evidence from animal models that vitamin D has the capacity to alter immune responses ^(11; 55; 56; 57). Vitamin D deficiency, for example, accelerates the development of experimental colitis in interleukin-10 (IL-10) knock-out mice ⁽¹²⁾ whereas treatment with dietary vitamin D and calcium appear to protect against the development of inflammation ⁽⁵⁸⁾. Downregulation of inflammatory cytokines, notably, tumour necrosis factor-alpha (TNF- α) and interferon gamma (IFN γ) and upregulation of IL10 has been demonstrated ^(58; 59) in response to vitamin D. Newer directions focus on effects of vitamin D as a late regulator of immune responses ⁽⁵⁶⁾ effects on autophagy ⁽⁶⁰⁾, on gut barrier integrity ^(61; 62) and on the gut microbiome ⁽⁶³⁾. Recently, Cantorna and Waddell ⁽⁵⁶⁾ proposed vitamin D as a late regulator of T-cell function, which acts to turn off chronically activated T-cells through the VDR - in contrast resting T-cells remain unresponsive to vitamin D because they do not express VDRs until late after activation ⁽⁵⁶⁾. Relevant to IBD, there is evidence that vitamin D deficiency may compromise the gastrointestinal mucosal barrier, whereas active vitamin D appears to promote epithelial integrity through upregulation of tight junction proteins zonula occludens-1 (ZO-1) and claudin-1^(61; 62). Some reports suggest changes in

the gut microbial composition in animal models ⁽⁶³⁾ in response to vitamin D. Despite immense interest in the gut microbiome ^(64; 65) this has yet to be investigated in response to vitamin D therapy in CD.

While some parallels exist, the degree to which the immune effects observed in animal models translate to human IBD is not fully understood. Consistent with experimental findings, Bartels et al ⁽¹⁵⁾ showed that the active form of vitamin D (1,25-dihydroxyvitamin D₃) increased IL-10 production and reduced IFN γ in T-cells derived from patients with CD. Similarly, we showed an inverse association between vitamin D deficiency and circulating IL-10 ⁽⁶⁶⁾ in a cross-sectional study of CD, however no association was noted for TNF- α . Arguable, this suggests that patients who are vitamin D deficient have lower IL-10 production and reduced anti-inflammatory capacity. In contrast, findings from other authors do not support this reduction in IL-10 in response to high dose vitamin D supplementation in CD ⁽⁴⁷⁾.

There is emerging, albeit inconsistent, mechanistic data from vitamin D intervention studies in CD. Increased IL-6 was reported, based on experiments on activated T-cells derived from patients (n=10) treated with 1200 IU in a RCT ⁽⁶⁷⁾. This finding appears at odds with putative anti-inflammatory effects and may reflect a paradoxical role for IL-6 in this clinical setting. Notably, in an open label study by Yang *et al* ⁽⁴⁷⁾, a series of inflammatory cytokines (TNF- α , IL-17, IL-10) remained unchanged in response to vitamin D supplementation, even though circulating levels of 25(OH)D of 100 nmol/L were achieved. Others hypothesise that therapeutic response of vitamin D may be mediated in part by effects on the intestinal barrier ^(68; 69). Preliminary results ⁽⁵⁰⁾ from our group suggest that vitamin D supplementation (2000 IU/day) may maintain intestinal permeability and barrier integrity in CD, but the findings from this pilot study require further investigation. Clearly, more mechanistic studies are needed to identify and confirm the mechanism by which vitamin may exert anti-inflammation effects in CD.

Vitamin D and Multiple Health Outcomes in IBD

Vitamin D may modify other health outcomes in IBD beyond bone and immune responses – this includes impacts on colorectal cancer risk and muscle strength. People with IBD are at increased risk of developing colorectal cancer due to chronic inflammation. In large population studies growing, though not entirely consistent, evidence suggests vitamin D as a

plausible candidate for colorectal cancer prevention ^(36; 37; 38; 70). Ananthakrishnan and colleagues, ⁽⁷¹⁾ recently reported an increased risk of colorectal cancer in a cohort (n = 2,809) of patients with IBD who were vitamin D deficient [25(OH)D <50 nmol/l]. Furthermore, a gradient-response was noted - each increment increase (2.5 nmol/L) in 25(OH)D concentration was associated with an 8% reduction in risk of colorectal cancer (Odds ratio, 0.92; CI, 0.88-0.96) ⁽⁷¹⁾. While this study suggests that vitamin D may have a chemoprevention role in IBD, the finding need to be replicated in further cohorts. Of note, this study enrolled patients who had an existing 25(OH)D measurement which may bias the study cohort; for example, patients in whom vitamin D assessment was clinically indicated may also have disease characteristics which confer an increased risk of cancer, independent of vitamin D status.

Reduced muscle strength has been documented in CD ⁽⁷²⁾ and while multifactorial in origin, one current theory implicates vitamin D status. An important focus of poor muscle function in CD is its role in exacerbating chronic fatigue, a common and often debilitating symptom reported by patients ⁽⁷³⁾. There is some evidence that vitamin D supplementation improves muscle strength ^(74; 75) at least in older adults. Equally, increasing circulating 25(OH)D greater than 75 nmol/L may result in reduced fatigue severity, although by undefined mechanisms ⁽⁷⁶⁾. In CD, preliminary findings from an RCT showed a significant reduction in self-reported fatigue in patients who achieved a 25(OH)D level greater than 75nmol/L in response to 2000 IU oral supplemental vitamin D ⁽⁷⁷⁾. Overall the findings remain inconclusive and further evidence would be required to understand if vitamin D plays a role in preserving muscle function, in cancer prevention and in other health outcomes in IBD.

Conclusion, Research Agenda and Future Directions

Several strands of evidence suggest vitamin D as a potential anti-inflammatory therapy for conditions such as IBD. Ultimately this potential will be judged based on a proven efficacy to induce or prolong disease remission as an adjunct to medical therapy. To date, intervention studies of vitamin D treatment for IBD are few, primarily conducted in CD and as yet provide insufficient evidence for translation to clinical practice.

For future studies it may be important to consider how vitamin D-specific and IBD-specific factors influence response to vitamin D treatment. For example, baseline vitamin D status, supplemental doses of vitamin D, subsequent circulating level of 25(OH)D achieved and magnitude of change from baseline. Moreover, other factors such as disease phenotype and

genotype and effects of IBD medications may impact on response. A clearer understanding of these and other factors such as the putative ‘therapeutic zone’ for vitamin D levels may help inform clinical consensus. If proven effective, vitamin D could offer a safe, inexpensive therapeutic strategy for IBD that provides additional bone benefits.

In the interim, at a minimum we should aim to prevent vitamin D deficiency in IBD. It seems reasonable to consider and investigate if there is an IBD-specific optimal 25(OH)D level. So what of the role of vitamin D as a novel anti-inflammatory therapy in IBD? This topic continues to attract interest; there is indeed some unavoidable ‘hype’, some ‘hope’ or promise from emerging scientific data, but a dearth of evidence. There is an ongoing need for high quality well-designed clinical trials and mechanistic studies to determine if the emerging anti-inflammatory effects for vitamin D translate into tangible clinical benefits for patients with chronic debilitating diseases such as IBD.

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Conflict of interest

None

Table 1: Overview of reported associations between circulating 25(OH)D levels and outcomes of disease activity in patients with CD

Reference	Year	Region	CD n	Disease Activity Score <i>CDAI / HBI</i>	Systemic inflammation <i>CRP</i>	Intestinal inflammation <i>Faecal Calprotectin</i>
Raftery <i>et al</i> ⁽⁴³⁾	2014	Ireland	85	X (CDAI)	X	√, inverse
Garg <i>et al</i> ⁽⁴²⁾	2013	Australia	40	X (HBI)	X	√ inverse
Jorgensen <i>et al</i> ⁽⁷⁸⁾	2013	Denmark	182	√ inverse (CDAI)	X	--
Kelly <i>et al</i> ⁽⁶⁶⁾	2011	Ireland	75	X (CDAI)	X	--
Ulitsky <i>et al</i> ⁽⁷⁹⁾ (total n~785)	2011	US	403	√ inverse (HBI)	--	---
Other Disease Outcomes						
Ananthakrishnan <i>et al</i> ⁽⁴⁴⁾	2013	US	1769	Low 25OHD and higher risk of surgery and hospitalisation		
Zator <i>et al</i> ⁽⁴⁵⁾	2013	US	74	Low 25(OH)D and loss of response to drug therapy		

CD, Crohn's disease; CRP, C-reactive protein; CDAI, Crohn's disease activity index; HBI, Harvey Bradshaw Index; Information in brackets “()” specifies the method of disease activity assessment used.

√, indicates a significant association between 25(OH)D levels and the marker in question, the direction of the association is stated as positive or inverse.

X, indicates no significant association reported between 25(OH)D levels and the marker in question.

Dash “-” indicates that the disease marker was not assessed/reported.

Table 2: Intervention studies of vitamin D supplementation to prevent relapse in adults with CD

Reference	Year	CD n	Disease activity at enrolment	Study Design	Dose of vitamin D	Duration of supplementation in months	Outcome Measure	Outcome
Jorgensen <i>et al</i> ⁽¹⁴⁾	2010	98	Inactive	RCT	1200 IU daily D ₃	12	Relapse at 1 year defined by CDAI	13% vs. 29% relapse for D ₃ vs. placebo (NS: p = 0.06)
Yang <i>et al</i> ⁽⁴⁷⁾	2013	18	Active (mild-moderate)	Open label	Titrated dose of D ₃ to achieve serum 25(OH)D 100 nmol/L	6	Change in CDAI	Significant reduction in CDAI score
Milhiller <i>et al</i> ⁽⁴⁶⁾	2009	37	Inactive	Open label	Active vitamin D and D ₃	12	Change in CDAI/ CRP	Significant reduction in CDAI score and CRP

CD, Crohn's disease; CRP, C-reactive protein; CDAI, Crohn's disease activity index; RCT, randomised controlled trial;

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