Journal of Crohn's and Colitis Advance Access published November 26, 2014

Title:

Vitamin D Therapy in Inflammatory Bowel Diseases who, in what form, and how much?

Tibor Hlavaty[1]

Anna Krajcovicova[1]

Juraj Payer[2]

Department of Internal Medicine, Division of Gastroenterology and Hepatology[1], Osteocentrum[2], University Hospital Bratislava, Ruzinovska

Key words:

Crohn's disease, ulcerative colitis, vitamin D supplementation, effectiveness, safety

Address for correspondence:

Assoc. Prof. Tibor Hlavaty, MD, PhD,

Department of Internal Medicine,

Division of Gastroenterology and Hepatology,

University Hospital Bratislava and Commenius University Bratislava

Ruzinovska 6, 826 06 Bratislava, Slovakia

Tel/Fax: +421 2 48234 905

E-mail: tibor.hlavaty2@gmail.com

Keywords: vitamin D, Ulcerative colitis, Crohn's disease

Word count (Body of manuscript excl tables and references): 5422

Tables: 3

Figures: 1

Accepted Manuscript

References: 153

Accepted Manuscript

Abstract

Background:

The north–south geographical gradient of inflammatory bowel disease (IBD) prevalence, the epidemiology of IBD and its genetic association with vitamin D receptor polymorphisms, and results in animal models suggest that vitamin D plays an important role in the pathogenesis of IBD.

Aims: The purpose of this review was to critically appraise the effectiveness and safety of vitamin D therapy in patients with IBD.

Methods: MEDLINE, Scopus and Google Scholar were searched from inception to 20th May 2014 using the terms 'Crohn's disease', 'ulcerative colitis' and 'vitamin D'.

Results: Vitamin D deficiency is common in patients with IBD. Limited clinical data suggest an association between low vitamin D concentration and increased disease activity in both ulcerative colitis (UC) and Crohn's disease (CD). To date, only two small open label trials and one randomised controlled trial have shown a positive effect of vitamin D supplementation on disease activity in patients with CD but not UC. An optimal vitamin D supplementation protocol for patients with IBD remains undetermined, but targeting serum 25(OH)D levels between 30 and 50 ng/ml appears safe and may have benefits for IBD disease activity. Depending on the baseline vitamin D serum concentration, ileal involvement in CD, body mass index and, perhaps, smoking status, daily vitamin D doses between 1800–10,000 IU/day are probably necessary.

Conclusion: Increasing pre-clinical and clinical evidence suggests a role for vitamin D deficiency in the development and severity of IBD. The possible therapeutic role of vitamin D in patients with IBD merits continued investigation.

Accepted Manuscript

Introduction

Inflammatory bowel diseases (IBD), which include ulcerative colitis (UC) and Crohn's disease (CD), are idiopathic chronic relapsing inflammatory conditions of the gastrointestinal tract. Although the precise etiologies of IBD are not known, both diseases are thought to result from dysregulation of the host immune response to commensal intestinal flora in genetically predisposed persons. The north–south gradient of IBD prevalence, its epidemiology, the genetic association of vitamin D receptor polymorphisms, and the many physiological studies of the effects of vitamin D on the immune system and mucosal barrier function suggest that vitamin D plays an important role in the pathogenesis of IBD [1-3]. Moreover, recent clinical evidence shows that vitamin D has promising clinical effects on disease activity, bone health, cancer prevention and depression. ***Therefore, we conducted a non-systematic review of published and in press articles, including reviews, meta-analyses, original studies, clinical trials and abstracts from scientific meetings to the extent that they were identified in the PubMed, Scopus, MEDLINE or GoogleScholar. The search terms included 'vitamin D' and 'Crohn's disease' or 'ulcerative colitis'.*** This review aims to explore the therapeutic potential of vitamin D in patients with CD and/or UC. Furthermore, based on current available evidence, we discuss the optimal target serum concentration, dosing, pharmacologic form and route of vitamin D supplementation.

1. Vitamin D

Vitamin D physiology

Vitamin D is a group of fat-soluble secosteroids with several hormonal functions in the human body. The physiology of vitamin D has been thoroughly reviewed [4]. The roles of vitamin D in calcium metabolism and healthy bone development have been recognised for more than a century. However, the discovery of the vitamin D receptor (VDR) in most tissues and cells of the body has provided new insights into the non-calcaemic functions of vitamin D, including cell proliferation, immunomodulation and cell differentiation [5-7].

The effects of vitamin D on the immune system are complex. Vitamin D receptor is abundantly expressed on immune cells, especially on activated T lymphocytes and dendritic cells. Vitamin D receptor is also expressed on antigen presenting cells, monocytes, macrophages, cytotoxic NK cells, and T and B lymphocytes [7 8]. Vitamin D has been shown to stimulate the production of T-regulatory lymphocytes and the expression of interleukin (IL)-4, IL-10 and transforming growth factor (TGF)-β, to inhibit the differentiation of CD4+ T lymphocytes into Th1 cells, and to suppress the effector functions of the latter [6 9]. These

Accepted Manuscript

immunomodulatory properties of vitamin D may explain the epidemiological associations between vitamin D status and a large number of immune-mediated diseases, including IBD, multiple sclerosis, rheumatoid arthritis, type I diabetes, systemic lupus erythematosus and psoriasis [10]. Vitamin D has also been associated with increased risks of infectious diseases, malignancies, transplant rejection and cardiovascular disease[11]. An overview of vitamin D sources, metabolism and effects on imunne system is outlined in Figure 1.

Figure 1

Vitamin D deficiency

Modern lifestyles appear to result in widespread vitamin D deficiency (~60%), especially at the end of winter [12 13]. The production of vitamin D3 depends on exposure to sunshine, the season of the year, latitude, clothing, skin pigmentation and the use of sunscreen. The intake of vitamin D in a typical western diet is limited (~40–100 IU/day) [13-15]. Therefore, a sun-deprived lifestyle is a major factor associated with vitamin D insufficiency.

2. Pre-clinical data linking vitamin D to IBD

Accumulating evidence from epidemiological, genetic, animal and in vitro studies has linked vitamin D to IBD (Table 1) [4].

Epidemiology

Epidemiologic studies have shown that vitamin D deficiency is highly prevalent among patients with IBD [16-19]. For example, vitamin D deficiency (<20 ng/ml) has been reported in 18–50% of patients with IBD at the end of summer and in 50–68% at the end of winter, with an additional ~35% of patients being vitamin D insufficient (20–29.9 ng/ml) [16-18 20-25].

Studies comparing vitamin D concentrations in IBD patients and healthy populations have yielded conflicting evidence. Although some of these studies have suggested that vitamin D concentrations are lower in IBD patients than in healthy controls [26 27], other studies have reported no difference [28-31]. Vitamin D deficiency in IBD can be aggravated by several factors, including decreased exposure to sunlight, decreased intestinal absorption of vitamin D in patients with CD and decreased oral intake [20 22 32-37].

Epidemiological evidence suggests that living at higher latitudes and/or being less exposed to sunlight is associated with a number of autoimmune diseases, including CD, UC, multiple sclerosis and diabetes mellitus type I [38-40]. This phenomenon, often called the north–south

Accepted Manuscript

gradient of IBD prevalence, has been observed in Europe as well as in North America [41 42]. For example, data from large observational population-based studies, the Nurses Health Studies I and II, with over 3,428,376 person-years of follow-up, showed that the incidence of CD and UC increased significantly with increasing latitude [43]. Compared with 30-year-old women residing in northern latitudes, the multivariate-adjusted hazard ratios (HRs) for 30-year-old women residing in southern latitudes were 0.48 (95% confidence interval (CI), 0.30–0.77) for CD and 0.62 (95% CI, 0.42–0.90) for UC. This north–south gradient is probably associated with higher levels of residential sun exposure in southern latitudes [42]. A large prospective population-based observational study of 91,870 healthy women aged 40–65 years up from 1990 onward identified 123 incident cases of IBD cases, including 45 with CD, 71 with UC and seven with indeterminatecolitis. Higher levels of residential sun exposure were associated with a significant decrease in the risk of CD (HR 0.49; 95% CI, 0.23–1.01; p = 0.04), but not of UC (HR, 1.21; 95% CI, 0.61–2.11) for the third versus the first tertile of mean daily ultraviolet radiation dose [44].

A third epidemiological investigation found that serum concentrations of vitamin D were lowest after winter [22 25]. This correlates with seasonal patterns in the incidence and flares of IBD, which peak during the spring season [45-49].

Lastly, smoking is a well recognised risk factor for CD and several studies have observed that smokers have lower vitamin D levels than non-smokers [18 50-52].

Genetic studies

Four vitamin D receptor polymorphisms have been associated with the risk of IBD: TaqI, BsmI, FokI and ApaI [53 54]. A recent meta-analysis of nine studies revealed that, in Asians, the ff genotype of FokI was associated with an increased risk of UC (odds ratio [OR] = 1.65; 95% CI, 1.11–2.45), the 'a' allele of ApaI appeared to protect against CD (OR = 0.81; 95% CI, 0.67–0.97) and the tt genotype of Taq1 increased the risk of CD in Europeans (OR = 1.23; 95% CI, 1.02–1.49) [2]. Another recent meta-analysis of 24 studies indicated that the AA genotype of ApaI significantly enhanced CD risk (AA versus aa: OR = 1.40; 95% CI, 1.05–1.88), the B allele of BsmI enhanced CD risk in East Asians (BB plus Bb versus bb: OR = 1.77; 95% CI, 1.14–2.74), and the T allele of TaqI was protective in Caucasians against CD (TT plus Tt versus tt: OR = 0.79; 95% CI, 0.63–1.00) and UC (T versus t: OR = 0.89, 95% CI, 0.80–0.99) [55].

Animal models

Accepted Manuscript

Vitamin D receptor agonists have shown benefical effects in animal models of many immune-mediated diseases, including IBD, rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and diabetes mellitus type I [56-58]. Studies in murine models of colitis showed the importance of vitamin D in the pathogenesis of IBD [17]. Vitamin D was shown to ameliorate the severity of experimental colitis in IL-10 deficient knockout (KO) mice, in that vitamin D-deficient IL-10 KO mice rapidly developed severe colitis, whereas vitamin D-sufficient IL-10 KO mice did not [59]. Moreover, supplementation with vitamin D ameliorated the severity of experimental colitis. In addition, mice deficient in both IL-10 and vitamin D receptors (double VDR/IL-10 KO) developed the most severe fulminant enterocolitis [58 60]. Mechanistically, these mice had reduced numbers of CD8alphaalpha intraepithelial lymphocytes, low levels of IL-10 and showed increased inflammatory responses to normally harmless commensal flora [61]. Furthermore, vitamin D was shown to inhibit several TNF-alpha-related genes in these animal models [62].

The findings in IL-10 KO mice were confirmed in dextrane sulphate sodium (DSS) and TNBS-induced colitis models [63-65]. The effects of vitamin D on immune system functions in these animal models were apparently influenced by dietary calcium [38]. Vitamin D-deficient mice on low-calcium diets developed severe IBD, whereas treatment of these mice with 1,25(OH)2D3 improved IBD symptoms; even more so in mice on high-calcium diets .

In vitro studies of vitamin D and innate immunity

Vitamin D signaling through its nuclear vitamin D receptor has emerged as a key regulator of innate immunity in humans. Vitamin D has been shown to up-regulate antimicrobial peptides, such as cathelicidins and beta-defensins, as well as autophagy processes [66-70]. Physiologic concentrations of 1,25(OH)2D inhibited the maturation of dendritic cells (DCs) by inhibiting activation markers such as MHC class II, CD40, CD80 and CD86, and up-regulating inhibitory molecules (ILT3), resulting in the maintenance of an immature and tolerogenic phenotype [71]. Following stimulation with 25(OH)D3, dendritic cells from CD patients displayed a reduced response to lipopolysaccharide and a diminished ability to activate T cells [72]. Interestingly vitamin D was found to robustly stimulate the expression of the NOD2 gene and protein in primary human monocytic and epithelial cells through distal enhancers in that gene [73]. Transcription of the NOD2 gene was stimulated directly by 1,25D-bound vitamin D receptor [74]. In addition, the vitamin D analogue TX 527 blocked the TNF-alpha-induced activation of NF-kappaB and the nuclear translocation of the latter, together with the degradation of IKB-alpha [75].

Accepted Manuscript

In vitro studies of vitamin D and adaptive immunity

The effects of 1,25(OH)2D on the adaptive immune system include decreasing the numbers of Th1/Th17 CD4+ T cells and cytokines, increasing regulatory T cells and down-regulating T cell-driven IgG production [7 76 77]. Furthermore, 1,25(OH)2D was shown to down-regulate IL-12 and augment IL-10 production by DCs, promoting a shift from a Th1 to a Th2 phenotype [71]. Supplementation with 140,000 IU/month vitamin D for 2 months in healthy subjects was associated with significantly increased Tregs in the peripheral circulation [78].

Vitamin D is also involved in the regulation of many cytokines including tumour necrosis factor alpha (TNF-α). Incubation of 1,25(OH)2D with DCs and T lymphocytes in vitro decreased the production of the pro-inflammatory cytokines TNF-α and interferon gamma (INF-γ) [79 80]. Incubation of peripheral blood mononuclear cells (PBMCs) isolated from UC patients with the vitamin D agonist calcitriol reduced IFN-γ and increased IL-10 production, whereas incubation of PBMCs from CD patients with calcitrol reduced TNF-α production [81]. Microarray analysis showed that 1,25(OH)2D3 down-regulated the expression of several TNF-α-related genes in colonic biopsy tissue [62]. Moreover, the vitamin D analogue KH 1060 synergised with anti-TNF-α treatment in decreasing TNF-α levels and inhibiting PBMC proliferation in samples from patients with IBD [82].

In vitro studies of vitamin D and mucosal barrier function

Vitamin D may also ameliorate IBD by playing a protective role in mucosal barrier homeostasis, maintaining the integrity of junction complexes and healing the colonic epithelium [63]. In cell cultures, 1,25(OH)2D3 markedly increased the expression of tight junction proteins and mRNA, and reduced mucosal permeability [83]. Vitamin D may also be involved in the physiological packaging of mucins in goblet cells [84]. Restoration of ionized calcium levels in VDR-ablated mice prevented altered mucin packaging, supporting the hypothesis that calcium is required for the physiological packaging of mucins in goblet cells. Interestingly, mice fed a vitamin D-deficient diet showed dysregulated colonic antimicrobial activity and had ~50-fold elevated levels of bacteria in colonic tissue [85].

3. Clinical evidence suggesting a role of vitamin D in the pathogenesis of IBD

Although considerable in vitro and animal experiments suggest that vitamin D has beneficial effects on the course of IBD, clinical data are scarce and are primarily

Accepted Manuscript

observational. Low vitamin D concentration has been associated with increased disease activity in patients with both UC and CD, although the evidence is conflicting [86 87]. To date, only two small open label trials, with 37 and 18 patients, respectively, and one randomised controlled trial with 104 patients, have analysed the effects of vitamin D supplementation on disease activity in patients with CD [88-90], but there have been no clinical trials in patients with UC.

Crohn's disease

Data supporting a clinical association between vitamin D deficiency and disease activity in CD are conflicting. A large study of 504 IBD patients, 403 with CD and 101 with UC, reported a correlation between vitamin D concentrations and disease activity (HBI, sIBDQ) in CD (regression coefficient, -2.21; 95% CI, -4.10 to -0.33) but not UC patients [17]. A retrospective study of 3217 patients with IBD, 55% with CD, found that plasma 25(OH)D concentrations <30 ng/mL in CD patients were associated with increased risks of surgery (OR 1.76; 95% CI, 1.24–2.51) and IBD-related hospitalisation (OR 2.07; 95% CI, 1.59–2.68) when compared with patients with 25(OH)D ≥30 ng/mL [24]. Furthermore, CD patients with initial 25(OH)D <30 ng/mL followed by subsequent normalisation had a reduced likelihood of surgery (OR 0.56; 95% CI, 0.32–0.98) compared with those who remained deficient. In a cross-sectional study of 182 CD patients and 62 healthy controls, serum 25(OH)D was inversely associated with disease activity [52]. Median vitamin D concentrations differed significantly among CD patients in remission (CDAI <150, vitamin D 25 ng/ml) and those with mild (CDAI 150–220, vitamin D 20 ng/ml) and moderately active (CDAI 220–400, vitamin D 8 ng/ml; $p < 0.01$) disease. A recent Chinese study on a cohort of 107 patients with CD found that 25(OH)D3 concentrations correlated negatively with disease severity ($r = -0.285$, $p = 0.030$) [91].

Several other small studies also confirmed the association of lower 25(OH)D concentrations and disease activity in CD patients, with correlation coefficients of approximately -0.4 [28 30 87 92 93]. A study examining whether pretreatment vitamin D status influences the durability of anti-TNF-α therapy in 74 patients with CD and 27 with UC found that patients with insufficient vitamin D ceased anti-TNF-α therapy earlier (HR, 2.13), due mainly to a loss of response (HR, 3.49), and that this effect was stronger for patients with CD (HR, 2.38) than UC [94]. By contrast, another study showed no correlation between CD activity and vitamin D status [26]. Thus, the link between vitamin D concentration and disease activity in patients with CD remains unclear [95].

Accepted Manuscript

Only a few clinical trials have assessed the effects of vitamin D in patients with CD. In a small open label trial of 35 CD patients in remission, treatment with 0.25 µg alfacalcidiol (1,25(OH)2D3) but not 1000 IU cholecalciferol (25(OH)D3) significantly reduced clinical activity, as measured by the CDAI, from 69 to 57, as well as the mean C-reactive protein concentration [89]. In a small open label trial of 18 CD patients with low to moderate disease activity, supplementation for 24 weeks with up to 5000 IU/day vitamin D3 significantly increased serum 25(OH)D3 concentrations and reduced mean CDAI scores from 230 ± 74 to 118 ± 66 ($p < 0.0001$) [90]. No significant adverse effects or signs of vitamin D toxicity were observed. A multicentre, randomised, double-blind placebo-controlled trial evaluating vitamin D maintenance therapy in 108 CD patients in clinical remission (CDAI score <150) found that treatment with 1200 IU/day vitamin D3 for 3 months improved vitamin D status significantly compared with placebo [88]. After a follow-up of 12 months, disease relapse rates were lower in the vitamin D than in the placebo group (29% versus 13%, $p = 0.06$).

Ulcerative colitis

Little is known about the correlation between UC activity and vitamin D status. High disease activity was reported to be more frequent in UC patients who were than were not vitamin D-deficient (<30 ng/ml) (68%, n = 19 versus 33%, n = 14; $p = 0.04$) [86]. A large retrospective study found that low plasma 25(OH)D was associated with increased risks of surgery and hospitalisation [24]. A recent Chinese study of 124 patients with UC found that 25(OH)D3 levels were negatively correlated with disease severity ($r = -0.371$, $p < 0.001$) [91].

By contrast, a large study of 504 IBD patients, including 101 with UC, reported no correlation between 25(OH)D concentrations and disease activity in UC patients [17]. Similarly, small Romanian (n = 33) and Iranian (n = 34) studies found that vitamin D levels did not correlate with disease activity, CRP concentration or erythrocyte sedimentation rate [92 93], and a study in 21 children newly diagnosed with UC found no correlation between UC activity and vitamin D concentration [26].

To date, no clinical trials have evaluated the effect of vitamin D supplementation on disease activity in patients with UC.

Other benefits of vitamin D supplementation

Vitamin D therapy may offer additional benefits to IBD patients, including improving bone health, reducing the risk of colorectal cancer and treating depressive symptoms [96].

Accepted Manuscript

Osteoporosis

The prevalence of low bone mineral density (BMD) is greater in patients with IBD than in healthy controls [97]. It has been estimated that 22–77% of patients with IBD have osteopenia and that 12–41% have osteoporosis [4 98]. A review of 20 randomised control trials (RCT) of 44,605 postmenopausal non-IBD women, evaluating the ability of vitamin D with or without calcium supplementation to prevent non-vertebral fractures and falls found that the higher dose (>380 IU/day), but not the lower dose, of vitamin D decreased hip fractures by 26% (RR, 0.74; CI, 0.61–0.88) and non-vertebral fractures by 23% (RR 0.77, CI 0.68–0.87) [99]. Optimal benefits were observed at the highest dose tested to date, 700 to 1000 IU per day or a mean 25(OH)D between 30–44 ng/ml. A meta-analysis of 19 randomised controlled trials of medical therapies for the treatment of low BMD in IBD patients concluded that data supporting the efficacy of calcium and current doses of vitamin D (400–800 IU/day) were insufficient [98]. ***This is in agreement with a recently published study by van Bodegraven et al, where the treatment with bisphosphonates concomitantly with vitamin D (800IU) and calcium (1000g) supplementation in osteopenic CD patients had a more beneficial effect on BMD than the supplementation of vitamin D and calcium alone [100] .***

Prevention of colorectal carcinoma

The first study suggesting an inverse relationship between exposure to sunshine and colorectal cancer (CRC) risk was published 35 years ago. A meta-analysis of five studies revealed that patients with serum 25(OH)D levels ≥33 ngl/l had a 50% lower risk of CRC than patients with 25(OH)D levels ≤12 nmol/l [101]. Furthermore, vitamin D intake of 1000 IU/day was associated with a 50% lower CRC risk than intake of <100 IU/day [102]. A review of prospective cohort data on CRC prevention suggested that an intake of 30–44 ng/ml 25(OH)D per day had the greatest benefits [99]. Analysis of the incidence of malignancies in 2809 IBD patients with a median follow-up of 11 years found that patients with vitamin D deficiency (~33%) were at increased risk of cancer (adjusted OR, 1.82; 95% CI, 1.25–2.65) compared with patients with sufficient vitamin D [103]. Each 1 ng/ml increase in 25(OH)D plasma concentration was associated with an 8% reduction in risk of CRC.

Depression

Depression and anxiety are common among IBD patients [104]. Depression can can add to disability in these patients, is a risk factor for disease flare-ups and can even impair the

Accepted Manuscript

effectiveness of medication [105] [106]. Several studies reported that vitamin D deficiency was significantly associated with mood disorders [107-109]. Supplementation with high (3000–6000 IU/d) but not low (400–600 IU/d) doses of vitamin D seemed to ameliorate the symptoms of depression [110-112].

Accepted Manuscript

4. Optimal supplementation protocol for IBD patients

Target vitamin D serum concentration

Vitamin D status is best evaluated by measuring the serum concentration of 25(OH)D. No consensus yet exists on the optimal serum levels of 25(OH)D, and health benefits may differ by target levels. 25(OH)D deficiencies are based largely on measurements of parathormone (PTH) concentrations and bone health. In most recent studies, 25(OH)D concentrations of >30 ng/mL (>75 nmol/L) are considered normal, with concentrations <20 ng/mL considered vitamin D deficient, and levels of 20–30 ng/mL considered vitamin D insufficient [69 113].

Physiological studies have shown maximal calcium and phosphate absorption at 25(OH)D serum concentration of 34 ng/ml (85 nmol/l), PTH increases at 25(OH)D levels below 31 ng/ml, and maximal genomic stability at levels of at least 36 ng/ml [114]. Two large patient cohorts showed minimal all-cause mortality rates at serum 25(OH)D concentrations of 40–48 ng/ml [115 116]. A meta-analysis of 18 RCTs assessing vitamin D supplementation for the prevention of falls and fractures found that serum 25(OH)D levels of about 30 to 44 ng/l provided optimal benefits for all investigated endpoints without increasing health risks [99]. In an interesting study from Hawaii, the highest 25(OH)D3 concentration recorded following natural exposure to ultraviolet B was 60 ng/ml [117].

The optimal vitamin D serum concentration in IBD remains undetermined. A recent study, however, found that disease activity was the lowest at vitamin D concentrations of 50–59 ng/ml in all analysed scenarios, including in patients with UC and CD, and in analyses performed during the summer and winter seasons [25].

Vitamin D supplementation dose

Despite considerable research, the daily intake of vitamin D needed by healthy subjects, as well as by special populations, remains undetermined [118].

Currently, the Institute of Medicine of the National Academies has recommended an intake of 600 IU/day (800 IU/day for those >70 yrs) vitamin D for the general population, with a tolerable upper limit of 4000 IU/day for all adults and children aged >8 years [119]. By contrast, the Endocrine Society recommended a daily intake for adults of 1500–2000 IU/day with an upper limit of 10,000 IU/day [113]. The daily allowance and upper limits for patients with increased vitamin D requirement due to malabsorption and vitamin D deficiencies have not been set.

Accepted Manuscript

It is generally believed that intake by healthy individuals of 100 IU/day vitamin D for 3 months increases serum 25(OH)D by approximately 1 ng/ml. However, this increase depends on several factors, including pretreatment serum 25(OH)D concentration, body fat mass (since vitamin D is a fat-soluble hormone), the formulation of vitamin D administered (D3>D2), the daily dose and the absorption capacity of the gastrointestinal tract [120]. Although a vitamin D3 dosage of 800 IU/d increased serum 25(OH)D levels to >20 ng/L in 97.5% of healthy postmenopausal women [121], vitamin D3 dosages required to attain serum concentrations of at least 30 ng/mL are less well-defined and may be as high as 1800 IU to 4000 IU per day [99]. Thus, dosages effective in raising serum 25(OH)D concentrations to the upper end of the normal range (30–60 ng/ml) are probably disproportionally compared with the dosages necessary to increase serum 25(OH)D concentrations just above normal (30 ng/ml).

Table 2 shows an overview of the relationship between vitamin D dose for at least 4 weeks and serum vitamin D concentrations in RCT for the prevention of osteoporosis [99]. A more detailed review of the largest trials is shown in Table 3.

There have been few consensus recommendations regarding vitamin D supplementation for patients with IBD. Studies have suggested that the ability to absorb vitamin D is about 30% lower in CD patients than in healthy individuals, suggesting that the daily doses recommended for the general population should be increased by a factor of 1.5 [32]. The European Crohn's and Colitis Organisation has recommended that patients on steroid therapy and those with reduced BMD receive vitamin D supplements but provided no detail on the dose [122]. The suggested vitamin D3 dose for IBD patients can also be based on baseline 25(OH)D and the presence of small bowel involvement, such that patients with serum 25(OH)D concentrations <4, 4–10, 10–16, 16–24 and 24 ng/ml should receive 5000, 4000, 3000, 2000 and 1000 IU/day, respectively [4]. These doses should be multiplied by 1.5–2 for small bowel involvement in CD patients and for obesity.

The oral dose of vitamin D (cholecalciferol) needed to raise the serum concentration to 40 ng/ml in CD patients was investigated by starting 18 patients on 1000 IU/day once daily [90]. Serum 25(OH)D3 levels were measured every 2 weeks, and vitamin D doses were increased by 1000 IU/day increments until serum 25(OH)D3 concentrations were above 40 ng/ml or until patients were taking 5000 IU/day vitamin D3. Fourteen of the eighteen patients required the maximum dose of 5000 IU. Morever, following administration of this dose for 24 weeks, half the patients did not reach the target vitamin D serum concentration of 40 ng/ml, although this protocol effectively and significantly increased mean serum 25(OH)D3 levels from 16 ±

Accepted Manuscript

10 ng/ml at baseline to 45 ± 19 ng/ml after 24 weeks. Another study, in paediatric IBD patients (mean age: 16 years), found that serum 25(OH)D3 levels increased from 0.8 to 1.1 ng/ml for every 10,000 IU of vitamin D2 and to 2 ng/ml for every 10,000 IU of vitamin D3 [123]. A recent prospective RCT involving 83 children aged 8–18 years with CD found that treatment with 2000 IU/day vitamin D3 for 6 months was more effective in raising vitamin D3 concentrations than 400 IU/day vitamin D3 (12 versus 2 ng/ml, $p < 0.001$) [124].

Several other retrospective trials investigated the effect of current supplementation doses on 25(OH)D serum concentrations. For example, 25(OH)D concentrations were significantly higher in CD patients who did than did not take 400–800 IU/day vitamin D (31 versus 22 ng/ml, $p < 0.01$) [52]. In addition, a recent study of 83 paediatric CD patients found that supplementation with 400 IU and 2000 IU vitamin D daily for 3 months increased mean serum 25(OH)D concentrations by 2.8 ng/ml and 16 ng/ml, respectively, over baseline [124]. In an Irish study, a supplement containing 200–400 IU/day vitamin D was insufficient in preventing vitamin D deficiency in patients with IBD [23]. In a Slovak study of 220 IBD patients, a vitamin D3 supplement (400–800 IU/day) significantly reduced the decline in serum vitamin D3 concentrations from summer to winter patients with CD (-0.7 versus -7.5 ng/ml, $p < 0.05$) [25].

Another factor that must be considered is the speed at which the target serum 25(OH)D concentration can be achieved. Little is known about the turnover of the vitamin D pool, although 25(OH)D is generally thought to have a half-life of 10–19 days [125], with four half-lives usually considered adequate to reach a steady state. Thus, vitamin D supplementation for 6–12 weeks should result in a steady state serum concentration. Most of this increase in serum 25(OH)D occurs early during the course of treatment with supplemental vitamin D3 [126].

The dosing schedule must also be considered. Data from clinical trials in non-IBD populations are inconsistent when comparing lower daily doses with higher weekly/monthly dosing of equipotent cumulative doses. A comparison in 40 elderly women of 400 IU twice daily (800 IU/day) with 97,333 IU of cholecalciferol every 4 months, yielding total doses of 292,000 IU in both groups, found that, after 12 months of treatment, 47% and 28%, respectively, achieved serum 25(OH)D levels of 30 ng/ml ($p < 0.0001$) [127]. However, compliance in these two groups was not compared.

Form of administraiton

Accepted Manuscript

Vitamin D can be administered in several oral or parenteral forms, including as cholecalciferol (vitamin D3), ergocalciferol (vitamin D2), 1- or 1,25-(di)hydroxyvitamin D3, or a vitamin D agonist (e.g., calcipotrol). ***These vitamin D supplements are available by prescription or without for a price of less than 10€ per month.*** Serum vitamin D concentration can also be increased by exposure to ultraviolet B radiation from sunshine or in a tanning bed.

The approach most often used to deliver vitamin D is oral supplementation with chole- or ergocalciferol. Several trials have compared the effectiveness of these two forms of vitamin D. In 69 rheumatology patients, a bolus of 300,000 IU of vitamin D3 had greater potency than equimolar vitamin D2, with a higher, sustained serum 25(OH)D response and greater suppression of PTH [128]. None of the patients who took vitamin D2 showed restoration of serum 25(OH)D to >20 ng/ml, whereas 100% and 80% of the patients who took vitamin D3 achieved 25(OH)D concentrations of >20 ng/L and >40 ng/ml, respectively. Several other studies demonstrated that the potency of vitamin D2 is less than one third that of vitamin D3, that vitamin D2 had a shorter duration of action than vitamin D3 and that vitamin D3 results in a 2- to 3-fold greater increase in vitamin D storage than vitamin D2 [129 130]. In an RCT of three vitamin D repletion regimens in 61 children with IBD, 6 weeks of oral supplementation with 2000 IU/day D2 (arm A), 2000 IU/day D3 (Arm B) and 50,000 IU/week D2 (Arm C) increased serum 25(OH)D serum concentrations by 9.3 ± 1.8 ng/ml (arm A), 16.4 ± 2.0 ng/ml (arm B) and 25.4 ± 2.5 ng/ml (arm C), respectively (A versus C, p = 0.0004; A versus B, p = 0.03) [123]. However, 62% and 25% of participants in arms B and C, respectively, did not achieve a serum 25(OH)D concentration above 30 ng/ml. A cumulative dose of 400,000 IU vitamin D2 or 220,000 IU vitamin D3 may be sufficient to achieve a serum concentration of 30 ng/ml. Interestingly, a small retrospective study of 108 veterans with IBD who received D3 were less likely than those who received D2 to use laboratory, pharmacy, radiology and fee-based services [131].

Alternatively, vitamin D can be administered in its hydroxylated forms, as 1,25-(OH)2 D3 and 1-(OH)D3, both of which are used to treat osteoporosis, or as vitamin D analogues, such as paricalcitol, maxacalcitol, alfacalciferol, doxecalciferol and calcipotrol. Although these compounds have not been assayed in the treatment of IBD, they were shown to be effective in small open label trials in psoriasis and rheumatoid arthritis [132-135].

Heliotherapy also increases serum vitamin D concentrations. Exposure of a human body in a bathing suit to one minimal erythema dose of sunlight has been reported to be equivalent to ingesting about 10,000–25,000 IU of vitamin D [136]. In a single case report, a CD patient

Accepted Manuscript

exposed to a tanning bed (UVB radiation 290–315 nm) while wearing a one-piece bathing suit for 10 minutes per day, 3 times per week for 4 weeks experienced an increase in serum 25(OH)D concentration from 7 to 32 ng/mL [137].

Route of application

The ability to absorb vitamin D is reduced in patients with CD, due either to inflammatory involvement of the proximal part of the small intestine or to reduced circulating bile acid pool after resection of the terminal ileum. Even patients with quiescent CD showed a mean 30% decrease in their ability to absorb vitamin D2 [32]. Vitamin D absorption is especially impaired in patients with previous ileal resections [35 36 138]. Treatment with oral cholecalciferol has been shown to be sufficient in patients with small or moderate resections (<300 cm), although other options, such as parenteral or oral 25-hydroxycholecalciferol supplementation, may be preferred in patients with severe short-bowel syndrome [35].

Topical applications of vitamin D have not yet been tested in patients with IBD. In animal models of IBD, the intrarectal application of the VDR agonists (BXL-62) and 1,25(OH)VD has been shown to be effective in ameliorating experimental DSS colitis [139].

Safety of vitamin D supplementation

Excess vitamin D can be toxic, as manifested by hypercalcemia. Excessive sunlight, however, does not lead to toxicity. A review of 25 RCTs of vitamin D supplementation for the prevention of osteoporotic fractures, falls, cardiovascular and oncologic diseases found that vitamin D supplementation was exceptionally safe [99]. Mean serum calcium levels did not correlate with oral vitamin D up to 100,000 IU/day or serum 25(OH)D concentrations up to 257 ng/ml. In all of these trials, only 24 patients experienced hypercalcemia, with most being asymptomatic and resolving after fasting. Hypocalcemia was reported in patients later diagnosed with primary hyperparathyroidism and in patients also taking calcium supplements of 1000–1500 mg per day. Of the 24 patients with hypercalcemia, 22 had serum 25(OH)D concentrations above 96 ng/ml. Even very large oral and parenteral loading doses of vitamin D3 (300,000–500,000 IU) were well tolerated and safe in small clinical trials [128 140].

Risk analyses found that 10,000 IU/day cholecalciferol is non-toxic in healthy adults, suggesting that it is safe as an upper limit of daily intake [141 142]. However, calcium absorption improves at higher serum 25(OH)D concentrations, suggesting the need to re-evaluate calcium supplements in patients taking >2000 IU/day vitamin D [99].

Accepted Manuscript

Of all controlled trials with vitamin D, an increased incidence of nephrolithiasis occurred only in the Women's Health Initiative (WHI) trial, in which patients received 400 IU/day vitamin D plus 1000 mg/day calcium, with an HR of 1.17 (95% CI, 1.02–1.34) [143]. However, the results of two large population-based cohorts suggests that nephrolithiasis was due to calcium, not vitamin D, intake [144 145].

5. Conclusion

Vitamin D supplementation and saturation of 25(OH)D3 reserves may be a novel therapeutic approach in patients with IBD, an approach that is simple, effective, safe and inexpensive. Preliminary pre-clinical and clinical data suggest that vitamin D has therapeutic potential in IBD, particularly in CD.

Patients with CD and perhaps those with UC who are vitamin D insufficient may benefit from vitamin D supplementation. The optimal concentration for patients with IBD remains unknown, but targeting serum 25(OH)D concentrations between 30 and 50 ng/ml appears safe and may have benefits for IBD disease activity, improving bone health, preventing CRC and alleviating depression. The dose needed to achieve these levels is probably much higher than that currently recommended daily allowances for healthy populations or supplementation doses for the treatment of reduced BMD. Depending on the baseline vitamin D serum concentration, ileal involvement in CD, body mass index and maybe also smoking status, daily doses of 1800–10,000 IU vitamin D are probably necessary. Cholecalciferol is the preferred oral form for supplementation and heliotherapy may also be effective.

Clinical trials are needed to determine the optimal therapeutic regime, including optimal target serum concentrations of vitamin D, and its forms, dosages and treatment protocols.

Accepted Manuscript

Accepted Manuscript

Supporting material

Contributors

All authors contributed to the critical revision of the manuscript and final approval of the version to be published. All authors agree to be accountable for all aspects of the work by ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding

This work was supported by a grant from Slovak APVV agency No. APVV-0672-11.

Competing interest

In the last 5 years, Tibor Hlavaty has served as a speaker, consultant or advisory board member for MSD, Abbvie, Hospira, Egis, Alfa Wasserman, Pfizer and Vifor. He has received scientific grants from Ferring Pharmaceuticals and unrestricted educational grants from MSD and Abbvie.

Accepted Manuscript

References

1. Raman M, Milestone AN, Walters JRF, Hart AL, Ghosh S. Vitamin D and gastrointestinal diseases: inflammatory bowel disease and colorectal cancer. *Therapeutic advances in gastroenterology* 2011;4(1):49-62.
2. Xue L-N, Xu K-Q, Zhang W, Wang Q, Wu J, Wang X-Y. Associations between vitamin D receptor polymorphisms and susceptibility to ulcerative colitis and Crohn's disease: a meta-analysis. *Inflammatory bowel diseases* 2013;19(1):54-60.
3. Ananthakrishnan AN, Khalili H, Higuchi LM, Bao Y, Korzenik JR, Giovannucci EL, et al. Higher predicted vitamin D status is associated with reduced risk of Crohn's disease. *Gastroenterology* 2012;142(3):482-9.
4. Garg M, Lubel JS, Sparrow MP, Holt SG, Gibson PR. Review article: vitamin D and inflammatory bowel disease--established concepts and future directions. *Alimentary pharmacology & therapeutics* 2012;36(4):324-44.
5. Haussler MR, Norman AW. Chromosomal receptor for a vitamin D metabolite. *Proc Natl Acad Sci U S A* 1969;62(1):155-62.
6. Nagpal S, Na S, Rathnachalam R. Noncalcemic actions of vitamin D receptor ligands. *Endocr Rev* 2005;26(5):662-87.
7. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. *J Mol Med (Berl)* 2010;88(5):441-50.
8. Miyaura C, Abe E, Nomura H, Nishii Y, Suda T. 1 alpha,25-Dihydroxyvitamin D3 suppresses proliferation of murine granulocyte-macrophage progenitor cells (CFU-C). *Biochem Biophys Res Commun* 1982;108(4):1728-33.
9. Bartels LE, Jørgensen SP, Agnholt J, Kelsen J, Hvas CL, Dahlerup JF. 1,25-dihydroxyvitamin D3 and dexamethasone increase interleukin-10 production in CD4+ T cells from patients with Crohn's disease. *Int Immunopharmacol* 2007;7(13):1755-64.
10. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004;80(6 Suppl):1678S-88S.
11. Holick MF. Vitamin D deficiency. *The New England journal of medicine* 2007;357(3):266-81.
12. Lips P. Worldwide status of vitamin D nutrition. *J Steroid Biochem Mol Biol* 2010;121(1-2):297-300.
13. Hintzpeter B, Mensink GBM, Thierfelder W, Müller MJ, Scheidt-Nave C. Vitamin D status and health correlates among German adults. *European journal of clinical nutrition* 2008;62(9):1079-89.
14. Peyrin-Biroulet L, Oussalah A, Bigard M-A. Crohn's disease: the hot hypothesis. *Med Hypotheses* 2009;73(1):94-6.
15. Vogelsang H, Klamert M, Resch H, Ferenci P. Dietary vitamin D intake in patients with Crohn's disease. *Wien Klin Wochenschr* 1995;107(19):578-81.
16. Pappa HM, Gordon CM, Saslowsky TM, Zholudev A, Horr B, Shih M-C, et al. Vitamin D status in children and young adults with inflammatory bowel disease. *Pediatrics* 2006;118(5):1950-61.
17. Ulitsky A, Ananthakrishnan AN, Naik A, Skaros S, Zadvornova Y, Binion DG, et al. Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity and quality of life. *JPEN. Journal of parenteral and enteral nutrition* 2011;35(3):308-16.

Accepted Manuscript

18. Gilman J, Shanahan F, Cashman KD. Determinants of vitamin D status in adult Crohn's disease patients, with particular emphasis on supplemental vitamin D use. *European journal of clinical nutrition* 2006;60(7):889-96.
19. Prosnitz AR, Leonard MB, Shults J, Zemel BS, Hollis BW, Denson LA, et al. Changes in vitamin D and parathyroid hormone metabolism in incident pediatric Crohn's disease. *Inflammatory bowel diseases* 2013;19(1):45-53.
20. McCarthy D, Duggan P, O'Brien M, Kiely M, McCarthy J, Shanahan F, et al. Seasonality of vitamin D status and bone turnover in patients with Crohn's disease. *Alimentary pharmacology & therapeutics* 2005;21(9):1073-83.
21. Levin AD, Wadhera V, Leach ST, Woodhead HJ, Lemberg DA, Mendoza-Cruz AC, et al. Vitamin D deficiency in children with inflammatory bowel disease. *Dig Dis Sci* 2011;56(3):830-6.
22. Bours PHA, Wielders JPM, Vermeijden JR, van de Wiel A. Seasonal variation of serum 25-hydroxyvitamin D levels in adult patients with inflammatory bowel disease. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2011;22(11):2857-67.
23. Suibhne TN, Cox G, Healy M, O'Morain C, O'Sullivan M. Vitamin D deficiency in Crohn's disease: prevalence, risk factors and supplement use in an outpatient setting. *J Crohns Colitis* 2012;6(2):182-8.
24. Ananthakrishnan AN, Cagan A, Gainer VS, Cai T, Cheng S-C, Savova G, et al. Normalization of plasma 25-hydroxy vitamin D is associated with reduced risk of surgery in Crohn's disease. *Inflammatory bowel diseases* 2013;19(9):1921-7.
25. Hlavaty T, Krajcovicova A, Koller T, Toth J, Nevidanska M, Huorka M, et al. Higher vitamin D serum concentration increases health related quality of life in patients with inflammatory bowel diseases. *World J Gastroenterol* 2014;20.
26. El-Matary W, Sikora S, Spady D. Bone mineral density, vitamin D, and disease activity in children newly diagnosed with inflammatory bowel disease. *Dig Dis Sci* 2011;56(3):825-9.
27. Silvennoinen J. Relationships between vitamin D, parathyroid hormone and bone mineral density in inflammatory bowel disease. *J Intern Med* 1996;239(2):131-7.
28. Tajika M, Matsuura A, Nakamura T, Suzuki T, Sawaki A, Kato T, et al. Risk factors for vitamin D deficiency in patients with Crohn's disease. *J Gastroenterol* 2004;39(6):527-33.
29. Harries AD, Brown R, Heatley RV, Williams LA, Woodhead S, Rhodes J. Vitamin D status in Crohn's disease: association with nutrition and disease activity. *Gut* 1985;26(11):1197-203.
30. Garg M, Rosella O, Lubel JS, Gibson PR. Association of circulating vitamin D concentrations with intestinal but not systemic inflammation in inflammatory bowel disease. *Inflammatory bowel diseases* 2013;19(12):2634-43.
31. de Bruyn JR, van Heeckeren R, Ponsioen CY, van den Brink GR, Löwenberg M, Bredenoord AJ, et al. Vitamin D deficiency in Crohn's disease and healthy controls: A prospective case-control study in the Netherlands. *J Crohns Colitis* 2014.
32. Farraye FA, Nimitphong H, Stucchi A, Dendrinos K, Boulanger AB, Vijjeswarapu A, et al. Use of a novel vitamin D bioavailability test demonstrates that vitamin D absorption is decreased in patients with quiescent Crohn's disease. *Inflammatory bowel diseases* 2011;17(10):2116-21.
33. Kumari M, Khazai NB, Ziegler TR, Nanes MS, Abrams SA, Tangpricha V. Vitamin D-mediated calcium absorption in patients with clinically stable Crohn's disease: a pilot study. *Mol Nutr Food Res* 2010;54(8):1085-91.

Accepted Manuscript

34. Vogelsang H, Schöfl R, Tillinger W, Ferenci P, Gangl A. 25-hydroxyvitamin D absorption in patients with Crohn's disease and with pancreatic insufficiency. *Wien Klin Wochenschr* 1997;109(17):678-82.
35. Leichtmann GA, Bengoa JM, Bolt MJ, Sitrin MD. Intestinal absorption of cholecalciferol and 25-hydroxycholecalciferol in patients with both Crohn's disease and intestinal resection. *Am J Clin Nutr* 1991;54(3):548-52.
36. Haderslev KV, Jeppesen PB, Sorensen HA, Mortensen PB, Staun M. Vitamin D status and measurements of markers of bone metabolism in patients with small intestinal resection. *Gut* 2003;52(5):653-8.
37. Jowett SL, Seal CJ, Phillips E, Gregory W, Barton JR, Welfare MR. Dietary beliefs of people with ulcerative colitis and their effect on relapse and nutrient intake. *Clin Nutr* 2004;23(2):161-70.
38. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. *Am J Clin Nutr* 2004;80(6 Suppl):1717S-20S.
39. Ponsonby A-L, McMichael A, van der Mei I. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. *Toxicology* 2002;181-182:71-8.
40. VanAmerongen BM, Dijkstra CD, Lips P, Polman CH. Multiple sclerosis and vitamin D: an update. *European journal of clinical nutrition* 2004;58(8):1095-109.
41. Schultz M, Butt AG. Is the north to south gradient in inflammatory bowel disease a global phenomenon? *Expert Rev Gastroenterol Hepatol* 2012;6(4):445-7.
42. Jussila A, Virta LJ, Salomaa V, Mäki J, Jula A, Färkkilä MA. High and increasing prevalence of inflammatory bowel disease in Finland with a clear North-South difference. *J Crohns Colitis* 2013;7(7):e256-62.
43. Khalili H, Huang ES, Ananthakrishnan AN, Higuchi L, Richter JM, Fuchs CS, et al. Geographical variation and incidence of inflammatory bowel disease among US women. *Gut* 2012;61(12):1686-92.
44. Jantchou P, Clavel-Chapelon F, Racine A, Kvaskoff M, Carbonnel F, Boutron-Ruault M-C. High residential sun exposure is associated with a low risk of incident Crohn's disease in the prospective E3N cohort. *Inflammatory bowel diseases* 2014;20(1):75-81.
45. Sonnenberg A, Jacobsen SJ, Wasserman IH. Periodicity of hospital admissions for inflammatory bowel disease. *The American journal of gastroenterology* 1994;89(6):847-51.
46. Aratari A, Papi C, Galletti B, Angelucci E, Viscido A, D'Ovidio V, et al. Seasonal variations in onset of symptoms in Crohn's disease. *Dig Liver Dis* 2006;38(5):319-23.
47. Lewis JD, Aberra FN, Lichtenstein GR, Bilker WB, Brensinger C, Strom BL. Seasonal variation in flares of inflammatory bowel disease. *Gastroenterology* 2004;126(3):665-73.
48. Tysk C, Järnerot G. Seasonal variation in exacerbations of ulcerative colitis. *Scandinavian journal of gastroenterology* 1993;28(1):95-6.
49. Disanto G, Chaplin G, Morahan JM, Giovannoni G, Hypponen E, Ebers GC, et al. Month of birth, vitamin D and risk of immune mediated disease: a case control study. *BMC Med* 2012;10(1):69.
50. Lorentzon M, Mellström D, Haug E, Ohlsson C. Smoking is associated with lower bone mineral density and reduced cortical thickness in young men. *J Clin Endocrinol Metab* 2007;92(2):497-503.
51. Siffledeen JS, Siminoski K, Steinhart H, Greenberg G, Fedorak RN. The frequency of vitamin D deficiency in adults with Crohn's disease. *Canadian journal of gastroenterology = Journal canadien de gastroenterologie* 2003;17(8):473-8.

Accepted Manuscript

52. Jørgensen SP, Hvas CL, Agnholt J, Christensen LA, Heickendorff L, Dahlerup JF. Active Crohn's disease is associated with low vitamin D levels. *J Crohns Colitis* 2013;7(10):e407-13.
53. Simmons JD, Mullighan C, Welsh KI, Jewell DP. Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. *Gut* 2000;47(2):211-4.
54. Naderi N, Farnood A, Habibi M, Derakhshan F, Balaii H, Motahari Z, et al. Association of vitamin D receptor gene polymorphisms in Iranian patients with inflammatory bowel disease. *J Gastroenterol Hepatol* 2008;23(12):1816-22.
55. Wang L, Wang ZT, Hu JJ, Fan R, Zhou J, Zhong J. Polymorphisms of the vitamin D receptor gene and the risk of inflammatory bowel disease: a meta-analysis. *Genet Mol Res* 2014;13(2):2598-610.
56. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc Natl Acad Sci U S A* 1996;93(15):7861-4.
57. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxycholecalciferol inhibits the progression of arthritis in murine models of human arthritis. *J Nutr* 1998;128(1):68-72.
58. Froicu M, Weaver V, Wynn TA, McDowell MA, Welsh JE, Cantorna MT. A crucial role for the vitamin D receptor in experimental inflammatory bowel diseases. *Mol Endocrinol* 2003;17(12):2386-92.
59. Cantorna MT, Munsick C, Bemiss C, Mahon BD. 1,25-Dihydroxycholecalciferol prevents and ameliorates symptoms of experimental murine inflammatory bowel disease. *J Nutr* 2000;130(11):2648-52.
60. Froicu M, Zhu Y, Cantorna MT. Vitamin D receptor is required to control gastrointestinal immunity in IL-10 knockout mice. *Immunology* 2006;117(3):310-8.
61. Yu S, Bruce D, Froicu M, Weaver V, Cantorna MT. Failure of T cell homing, reduced CD4/CD8alphaalpha intraepithelial lymphocytes, and inflammation in the gut of vitamin D receptor KO mice. *Proc Natl Acad Sci U S A* 2008;105(52):20834-9.
62. Zhu Y, Mahon BD, Froicu M, Cantorna MT. Calcium and 1 alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. *Eur J Immunol* 2005;35(1):217-24.
63. Kong J, Zhang Z, Musch MW, Ning G, Sun J, Hart J, et al. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *Am J Physiol Gastrointest Liver Physiol* 2008;294(1):G208-16.
64. Froicu M, Cantorna MT. Vitamin D and the vitamin D receptor are critical for control of the innate immune response to colonic injury. *BMC Immunol* 2007;8:5.
65. Verlinden L, Leyssens C, Beullens I, Marcelis S, Mathieu C, De Clercq P, et al. The vitamin D analog TX527 ameliorates disease symptoms in a chemically induced model of inflammatory bowel disease. *J Steroid Biochem Mol Biol* 2013;136:107-11.
66. Wang T-T, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004;173(5):2909-12.
67. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006;311(5768):1770-3.
68. Liu PT, Schenk M, Walker VP, Dempsey PW, Kanchanapoomi M, Wheelwright M, et al. Convergence of IL-1beta and VDR activation pathways in human TLR2/1-induced antimicrobial responses. *PLoS One* 2009;4(6):e5810.
69. Mouli VP, Ananthakrishnan AN. Review article: vitamin D and inflammatory bowel diseases. *Alimentary pharmacology & therapeutics* 2014;39(2):125-36.

Accepted Manuscript

70. Adams JS, Ren S, Liu PT, Chun RF, Lagishetty V, Gombart AF, et al. Vitamin d-directed rheostatic regulation of monocyte antibacterial responses. *J Immunol* 2009;182(7):4289-95.
71. Penna G, Roncari A, Amuchastegui S, Daniel KC, Berti E, Colonna M, et al. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+ regulatory T cells by 1,25-dihydroxyvitamin D3. *Blood* 2005;106(10):3490-7.
72. Bartels LE, Jørgensen SP, Bendix M, Hvas CL, Agnholt J, Agger R, et al. 25-Hydroxy vitamin D3 modulates dendritic cell phenotype and function in Crohn's disease. *Inflammopharmacology* 2013;21(2):177-86.
73. Wang T-T, Dabbas B, Laperriere D, Bitton AJ, Soualhine H, Tavera-Mendoza LE, et al. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. *J Biol Chem* 2010;285(4):2227-31.
74. Verway M, Behr MA, White JH. Vitamin D, NOD2, autophagy and Crohn's disease. *Expert Rev Clin Immunol* 2010;6(4):505-8.
75. Stio M, Martinesi M, Bruni S, Treves C, Mathieu C, Verstuyf A, et al. The Vitamin D analogue TX 527 blocks NF-kappaB activation in peripheral blood mononuclear cells of patients with Crohn's disease. *J Steroid Biochem Mol Biol* 2007;103(1):51-60.
76. Guillot X, Semerano L, Saidenberg-Kermanac'h N, Falgarone G, Boissier M-C. Vitamin D and inflammation. *Joint Bone Spine* 2010;77(6):552-7.
77. Gregori S, Giarratana N, Smiroldo S, Uskokovic M, Adorini L. A 1alpha,25-dihydroxyvitamin D(3) analog enhances regulatory T-cells and arrests autoimmune diabetes in NOD mice. *Diabetes* 2002;51(5):1367-74.
78. Prietl B, Pilz S, Wolf M, Tomaschitz A, Obermayer-Pietsch B, Graninger W, et al. Vitamin D supplementation and regulatory T cells in apparently healthy subjects: vitamin D treatment for autoimmune diseases? *Isr Med Assoc J* 2010;12(3):136-9.
79. Müller K, Haahr PM, Diamant M, Rieneck K, Kharazmi A, Bendtzen K. 1,25-Dihydroxyvitamin D3 inhibits cytokine production by human blood monocytes at the post-transcriptional level. *Cytokine* 1992;4(6):506-12.
80. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O'Garra A. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. *J Immunol* 2001;167(9):4974-80.
81. Ardizzone S, Cassinotti A, Trabattoni D, Manzionna G, Rainone V, Bevilacqua M, et al. Immunomodulatory effects of 1,25-dihydroxyvitamin D3 on TH1/TH2 cytokines in inflammatory bowel disease: an in vitro study. *Int J Immunopathol Pharmacol* 2009;22(1):63-71.
82. Stio M, Martinesi M, Bruni S, Treves C, d'Albasio G, Bagnoli S, et al. Interaction among vitamin D(3) analogue KH 1060, TNF-alpha, and vitamin D receptor protein in peripheral blood mononuclear cells of inflammatory bowel disease patients. *Int Immunopharmacol* 2006;6(7):1083-92.
83. Zhao H, Zhang H, Wu H, Li H, Liu L, Guo J, et al. Protective role of 1,25(OH)2 vitamin D3 in the mucosal injury and epithelial barrier disruption in DSS-induced acute colitis in mice. *BMC Gastroenterol* 2012;12:57.
84. Paz HB, Tisdale AS, Danjo Y, Spurr-Michaud SJ, Argüeso P, Gipson IK. The role of calcium in mucin packaging within goblet cells. *Experimental eye research* 2003;77(1):69-75.
85. Lagishetty V, Misharin AV, Liu NQ, Lisse TS, Chun RF, Ouyang Y, et al. Vitamin D deficiency in mice impairs colonic antibacterial activity and predisposes to colitis. *Endocrinology* 2010;151(6):2423-32.

Accepted Manuscript

86. Blanck S, Aberra F. Vitamin d deficiency is associated with ulcerative colitis disease activity. *Dig Dis Sci* 2013;58(6):1698-702.
87. Joseph AJ, George B, Pulimood AB, Seshadri MS, Chacko A. 25 (OH) vitamin D level in Crohn's disease: association with sun exposure & disease activity. *The Indian journal of medical research* 2009;130(2):133-7.
88. Jørgensen SP, Agnholt J, Glerup H, Lyhne S, Villadsen GE, Hvas CL, et al. Clinical trial: vitamin D3 treatment in Crohn's disease - a randomized double-blind placebo-controlled study. *Alimentary Pharmacology & Therapeutics* 2010;32(3):377-83.
89. Miheller P, Muzes G, Hritz I, Lakatos G, Pregun I, Lakatos PL, et al. Comparison of the effects of 1,25 dihydroxyvitamin D and 25 hydroxyvitamin D on bone pathology and disease activity in Crohn's disease patients. *Inflammatory bowel diseases* 2009;15(11):1656-62.
90. Yang L, Weaver V, Smith JP, Bingaman S, Hartman TJ, Cantorna MT. Therapeutic effect of vitamin d supplementation in a pilot study of Crohn's patients. *Clin Transl Gastroenterol* 2013;4:e33.
91. Tan B, Li P, Lv H, Li Y, Wang O, Xing XP, et al. Vitamin D levels and bone metabolism in Chinese adult patients with inflammatory bowel disease. *J Dig Dis* 2014;15(3):116-23.
92. Dumitrescu G, Mihai C, Dranga M, Prelipcean CC. Serum 25-hydroxyvitamin D concentration and inflammatory bowel disease characteristics in Romania. *World J Gastroenterol* 2014;20(9):2392-6.
93. Hassan V, Hassan S, Seyed-Javad P, Ahmad K, Asieh H, Maryam S, et al. Association between Serum 25 (OH) Vitamin D Concentrations and Inflammatory Bowel Diseases (IBDs) Activity. *Med J Malaysia* 2013;68(1):35.
94. Zator ZA, Cantu SM, Konijeti GG, Nguyen DD, Sauk J, Yajnik V, et al. Pretreatment 25-Hydroxyvitamin D Levels and Durability of Anti-Tumor Necrosis Factor-α Therapy in Inflammatory Bowel Diseases. *JPEN. Journal of parenteral and enteral nutrition* 2013.
95. Palmer MT, Weaver CT. Linking vitamin d deficiency to inflammatory bowel disease. *Inflammatory bowel diseases* 2013;19(10):2245-56.
96. Narula N, Marshall JK. Management of inflammatory bowel disease with vitamin D: beyond bone health. *J Crohns Colitis* 2012;6(4):397-404.
97. Miznerova E, Hlavaty T, Koller T, Toth J, Holociova K, Huorka M, et al. The prevalence and risk factors for osteoporosis in patients with inflammatory bowel disease. *Bratisl Lek Listy* 2013;114(8):439-45.
98. Melek J, Sakuraba A. Efficacy and safety of medical therapy for low bone mineral density in patients with inflammatory bowel disease: a meta-analysis and systematic review. *Clin Gastroenterol Hepatol* 2014;12(1):32-44.e5.
99. Bischoff-Ferrari HA, Shao A, Dawson-Hughes B, Hathcock J, Giovannucci E, Willett WC. Benefit-risk assessment of vitamin D supplementation. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2010;21(7):1121-32.
100. van Bodegraven AA, Bravenboer N, Witte BI, Dijkstra G, van der Woude CJ, Stokkers PC, et al. Treatment of bone loss in osteopenic patients with Crohn's disease: a double-blind, randomised trial of oral risedronate 35 mg once weekly or placebo, concomitant with calcium and vitamin D supplementation. *Gut* 2013:gutjnl-2013-305523.

Accepted Manuscript

101. Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, et al. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. *Am J Prev Med* 2007;32(3):210-6.
102. Gorham ED, Garland CF, Garland FC, Grant WB, Mohr SB, Lipkin M, et al. Vitamin D and prevention of colorectal cancer. *J Steroid Biochem Mol Biol* 2005;97(1-2):179-94.
103. Ananthakrishnan AN, Cheng S-C, Cai T, Cagan A, Gainer VS, Szolovits P, et al. Association Between Reduced Plasma 25-Hydroxy Vitamin D and Increased Risk of Cancer in Patients With Inflammatory Bowel Diseases. *Clin Gastroenterol Hepatol* 2013.
104. Farrokhyar F, Marshall JK, Easterbrook B, Irvine EJ. Functional gastrointestinal disorders and mood disorders in patients with inactive inflammatory bowel disease: prevalence and impact on health. *Inflammatory bowel diseases* 2006;12(1):38-46.
105. Mardini HE, Kip KE, Wilson JW. Crohn's disease: a two-year prospective study of the association between psychological distress and disease activity. *Dig Dis Sci* 2004;49(3):492-7.
106. Persoons P, Vermeire S, Demyttenaere K, Fischler B, Vandenberghe J, Van Oudenhove L, et al. The impact of major depressive disorder on the short- and long-term outcome of Crohn's disease treatment with infliximab. *Alimentary pharmacology & therapeutics* 2005;22(2):101-10.
107. Ganji V, Milone C, Cody MM, McCarty F, Wang YT. Serum vitamin D concentrations are related to depression in young adult US population: the Third National Health and Nutrition Examination Survey. *Int Arch Med* 2010;3(3):29.
108. Wilkins CH, Sheline YI, Roe CM, Birge SJ, Morris JC. Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. *Am J Geriatr Psychiatry* 2006;14(12):1032-40.
109. Armstrong DJ, Meenagh GK, Bickle I, Lee ASH, Curran E-S, Finch MB. Vitamin D deficiency is associated with anxiety and depression in fibromyalgia. *Clin Rheumatol* 2007;26(4):551-4.
110. Harris S, Dawson-Hughes B. Seasonal mood changes in 250 normal women. *Psychiatry Res* 1993;49(1):77-87.
111. Jorde R, Sneve M, Figenschau Y, Svartberg J, Waterloo K. Effects of vitamin D supplementation on symptoms of depression in overweight and obese subjects: randomized double blind trial. *J Intern Med* 2008;264(6):599-609.
112. Vieth R, Kimball S, Hu A, Walfish PG. Randomized comparison of the effects of the vitamin D3 adequate intake versus 100 mcg (4000 IU) per day on biochemical responses and the wellbeing of patients. *Nutrition journal* 2004;3:8.
113. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96(7):1911-30.
114. Schwalfenberg GK, Genuis SJ, Hiltz MN. Addressing vitamin D deficiency in Canada: a public health innovation whose time has come. *Public Health* 2010;124(6):350-9.
115. Dobnig H, Pilz S, Scharnagl H, Renner W, Seelhorst U, Wellnitz B, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin d levels with all-cause and cardiovascular mortality. *Arch Intern Med* 2008;168(12):1340-9.
116. Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* 2008;168(15):1629-37.
117. Binkley N, Novotny R, Krueger D, Kawahara T, Daida YG, Lensmeyer G, et al. Low vitamin D status despite abundant sun exposure. *J Clin Endocrinol Metab* 2007;92(6):2130-5.

Accepted Manuscript

118. Binkley N, Wiebe D. Clinical controversies in vitamin D: 25(OH)D measurement, target concentration, and supplementation. *J Clin Densitom* 2013;16(4):402-8.
119. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US), 2011.
120. Barger-Lux MJ, Heaney RP, Dowell S, Chen TC, Holick MF. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 1998;8(3):222-30.
121. Gallagher JC, Sai A, Templin T, Smith L. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. *Ann Intern Med* 2012;156(6):425-37.
122. Van Assche G, Dignass A, Reinisch W, van der Woude CJ, Sturm A, De Vos M, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. *J Crohns Colitis* 2010;4(1):63-101.
123. Pappa HM, Mitchell PD, Jiang H, Kassiff S, Filip-Dhima R, DiFabio D, et al. Treatment of vitamin D insufficiency in children and adolescents with inflammatory bowel disease: a randomized clinical trial comparing three regimens. *J Clin Endocrinol Metab* 2012;97(6):2134-42.
124. Wingate KE, Jacobson K, Issenman R, Carroll M, Barker C, Israel D, et al. 25-Hydroxyvitamin D Concentrations in Children with Crohn's Disease Supplemented with Either 2000 or 400 IU Daily for 6 Months: A Randomized Controlled Study. *J Pediatr* 2014.
125. Vicchio D, Yergey A, O'Brien K, Allen L, Ray R, Holick M. Quantification and kinetics of 25-hydroxyvitamin D3 by isotope dilution liquid chromatography/thermospray mass spectrometry. *Biol Mass Spectrom* 1993;22(1):53-8.
126. Lips P, Wiersinga A, van Ginkel FC, Jongen MJ, Netelenbos JC, Hackeng WH, et al. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. *J Clin Endocrinol Metab* 1988;67(4):644-50.
127. Pekkarinen T, Välimäki V-V, Aarum S, Turpeinen U, Hämäläinen E, Löyttyniemi E, et al. The same annual dose of 292000 IU of vitamin D (cholecalciferol) on either daily or four monthly basis for elderly women: 1-year comparative study of the effects on serum 25(OH)D concentrations and renal function. *Clin Endocrinol (Oxf)* 2010;72(4):455-61.
128. Leventis P, Kiely PDW. The tolerability and biochemical effects of high-dose bolus vitamin D2 and D3 supplementation in patients with vitamin D insufficiency. *Scand J Rheumatol* 2009;38(2):149-53.
129. Armas LAG, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. *J Clin Endocrinol Metab* 2004;89(11):5387-91.
130. Heaney RP, Recker RR, Grote J, Horst RL, Armas LAG. Vitamin D(3) is more potent than vitamin D(2) in humans. *J Clin Endocrinol Metab* 2011;96(3):E447-52.
131. Youssef D, Bailey B, Atia A, El-Abbassi A, Manning T, Peiris AN. Differences in outcomes between cholecalciferol and ergocalciferol supplementation in veterans with inflammatory bowel disease. *Geriatr Gerontol Int* 2012;12(3):475-80.
132. Smith EL, Pincus SH, Donovan L, Holick MF. A novel approach for the evaluation and treatment of psoriasis. Oral or topical use of 1,25-dihydroxyvitamin D3 can be a safe and effective therapy for psoriasis. *Journal of the American Academy of Dermatology* 1988;19(3):516-28.

Accepted Manuscript

133. Andjelkovic Z, Vojinovic J, Pejnovic N, Popovic M, Dujic A, Mitrovic D, et al. Disease modifying and immunomodulatory effects of high dose 1 alpha (OH) D3 in rheumatoid arthritis patients. *Clin Exp Rheumatol* 1999;17(4):453-6.
134. Fogh K, Kragballe K. Recent developments in vitamin D analogs. *Curr Pharm Des* 2000;6(9):961-72.
135. Ruzicka T, Lorenz B. Comparison of calcipotriol monotherapy and a combination of calcipotriol and betamethasone valerate after 2 weeks' treatment with calcipotriol in the topical therapy of psoriasis vulgaris: a multicentre, double-blind, randomized study. *Br J Dermatol* 1998;138(2):254-8.
136. Holick MF. Sunlight "D"ilemma: risk of skin cancer or bone disease and muscle weakness. *Lancet* 2001;357(9249):4-6.
137. Koutkia P, Lu Z, Chen TC, Holick MF. Treatment of vitamin D deficiency due to Crohn's disease with tanning bed ultraviolet B radiation. *Gastroenterology* 2001;121(6):1485-8.
138. Driscoll RH, Meredith SC, Sitrin M, Rosenberg IH. Vitamin D deficiency and bone disease in patients with Crohn's disease. *Gastroenterology* 1982;83(6):1252-8.
139. Laverny G, Penna G, Vetrano S, Correale C, Nebuloni M, Danese S, et al. Efficacy of a potent and safe vitamin D receptor agonist for the treatment of inflammatory bowel disease. *Immunol Lett* 2010;131(1):49-58.
140. Bacon CJ, Gamble GD, Horne AM, Scott MA, Reid IR. High-dose oral vitamin D3 supplementation in the elderly. *Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA* 2009;20(8):1407-15.
141. Hathcock JN, Shao A, Vieth R, Heaney R. Risk assessment for vitamin D. *The American Journal of Clinical Nutrition* 2007;85(1):6-18.
142. Vieth R. Vitamin D toxicity, policy, and science. *J Bone Miner Res* 2007;22 Suppl 2:V64-8.
143. Jackson RD, LaCroix AZ, Gass M, Wallace RB, Robbins J, Lewis CE, et al. Calcium plus vitamin D supplementation and the risk of fractures. *The New England journal of medicine* 2006;354(7):669-83.
144. Taylor EN, Stampfer MJ, Curhan GC. Dietary factors and the risk of incident kidney stones in men: new insights after 14 years of follow-up. *J Am Soc Nephrol* 2004;15(12):3225-32.
145. Curhan GC, Willett WC, Knight EL, Stampfer MJ. Dietary factors and the risk of incident kidney stones in younger women: Nurses' Health Study II. *Arch Intern Med* 2004;164(8):885-91.

Accepted Manuscript

Table 1: Summary of epidemiological, genetic and in vitro evidence for the role of vitamin D in the pathogenesis of IBD

Epidemiology	North–south gradient of IBD prevalence in North America and Europe [41 42]. Low prevalence in countries near equator [43]. Low predicted vitamin D level increases risk of CD and UC [44]. Seasonality: UC and CD flares peak at the end of low-sunshine season [45-49]. Seasonality: Risk of UC higher in children born to mothers with lower predicted sunshine exposure in second trimester [49].
Genetic studies	Asians: ff genotype of FokI associated with UC, ApaI 'a' allele protective factor for CD [2]. Caucasians: ApaI tt genotype associated with CD [55].
Animal models	VDR KO mice develop severe colitis in several models of IBD colitis (IL-10 KO, DSS and TNBS) [58-60 63-65]. Supplementation with vitamin D ameliorates experimental colitis in several IBD models [59]. Mice deficient in dietary vitamin D have decreased colonic antimicrobial activity and elevated levels of bacteria in colonic tissue [61 62 85].
In vitro studies	Vitamin D promotes maintenance of epithelial barriers (tight junctions and packaging of mucin in goblet cells) [63 83 84]. Vitamin D enhances protective innate immune responses (NOD2 expression, cathelicid and defensin production) [66-73]. Vitamin D regulates adaptive immune responses (increased Th2/Treg; decreased Th1/Th17 CD4+; inhibition of maturation of DCs; down-regulation of TNF-α, IL12, IL-17, IL-21 and IFN-γ; up-regulation of IL6, IL10 and FoxP3 [7 71 76 77 79-81].

Accepted Manuscript

Table 2: Effect of vitamin D supplementation during randomised clinical trials for fracture prevention on serum 25(OH)D concentrations

Vitamin D dose (IU/day)	Number of RCTs	Mean serum vitamin D concentration achieved (ng/ml) [range]
400	3	23 [18–26]
700 *	1	40 *
800	9	30 [24–42]
2000	7	35 [28–41]
4000	3	48 [34–64]
5720–7600	4	51 [48–59]

Data based on [99].

* The vitamin D concentration before supplementation was high in this trial (31 ng/ml).

Accepted Manuscript

Table 3: Effects of vitamin D supplementation on serum vitamin concentration in selected trials

Study	Population	Vitamin D3 dose (IU/day unless stated otherwise)	Duration of supplementation	Baseline vitamin D (ng/ml)	Follow-up vitamin D (ng/ml)
Lyons 2007 [145]	3440 elderly people (2724 women)	A: 100,000 IU every 4 months B: placebo	36 months	-	A: 32.17 (20 months) B: 21.69 *
Pfeifer (2009) [146]	242 healthy seniors	A: 800 IU + 1 g Ca B: placebo + 1 g Ca	12 + 8 months free follow-up	A: 22.1 ± 7.2 B: 21.7 ± 7.6	A: 33.7 ± 7.2 B: 22.9 ± 8.0
Trivedi (2003) [147]	2686 (2037 men)	A: 100,000 IU every 4 months B: placebo	48 months	-	A: 29.83 ± 8.3 B: 21.45 ± 8.5
Chapuy (2002) [148]	583 elderly women	A: 800 IU + 1.2 g Ca B: placebo	24 months	A: 8.55 ± 5.3 B: 9.16 ± 6.9	A: 31.12 B: 6.02
Meyer (2002) [149]	1144 elderly	A: 400 IU B: placebo	12 months	A: 18.88 ± 10.4 B: 20.48 ± 13.25	A: 25.7 ± 8.4 B: 18.47 ± 8.03
Grant (2004) [150]	5292 elderly (measured on 60 patients)	A: 800 IU B: 1 g Ca C: 800 IU + 1 g Ca D: placebo	12 months	15.2 ± 6.5	A: 24.9 ± 8.7 B: 16.6 ± 5.7 C: 24.8 ± 6.9 D: 18.3 ± 7.2
Lips (1996) [151]	2578 adults	A: 400 IU B: placebo	12 months	A: 10.84 B: 10.44	A: 24.9 B: 9.23
Vieth (2001) [152]	61 healthy	A: 1000 IU B: 4000 IU	3 months	A: 17.4 ± 6.7 B: 15.2 ± 5.4	A: 27.6 ± 6.8 B: 38.7 ± 5.9
Broe (2007) [153]	124 elderly	A: 200 IU B: 400 IU C: 600 IU D: 800 IU E: 0	5 months	A:17.8 ± 9.2 B: 20.7 ± 11.6 C:16.5 ± 7.4 D:21.4 ± 9.2 E:21.2 ± 11.4	A: 24.5 ± 8.29 B: 21.89 ± 9.22 C: 24.47 ± 8.78 D: 29.82 ± 6.26 E: 24.12 ± 13.04

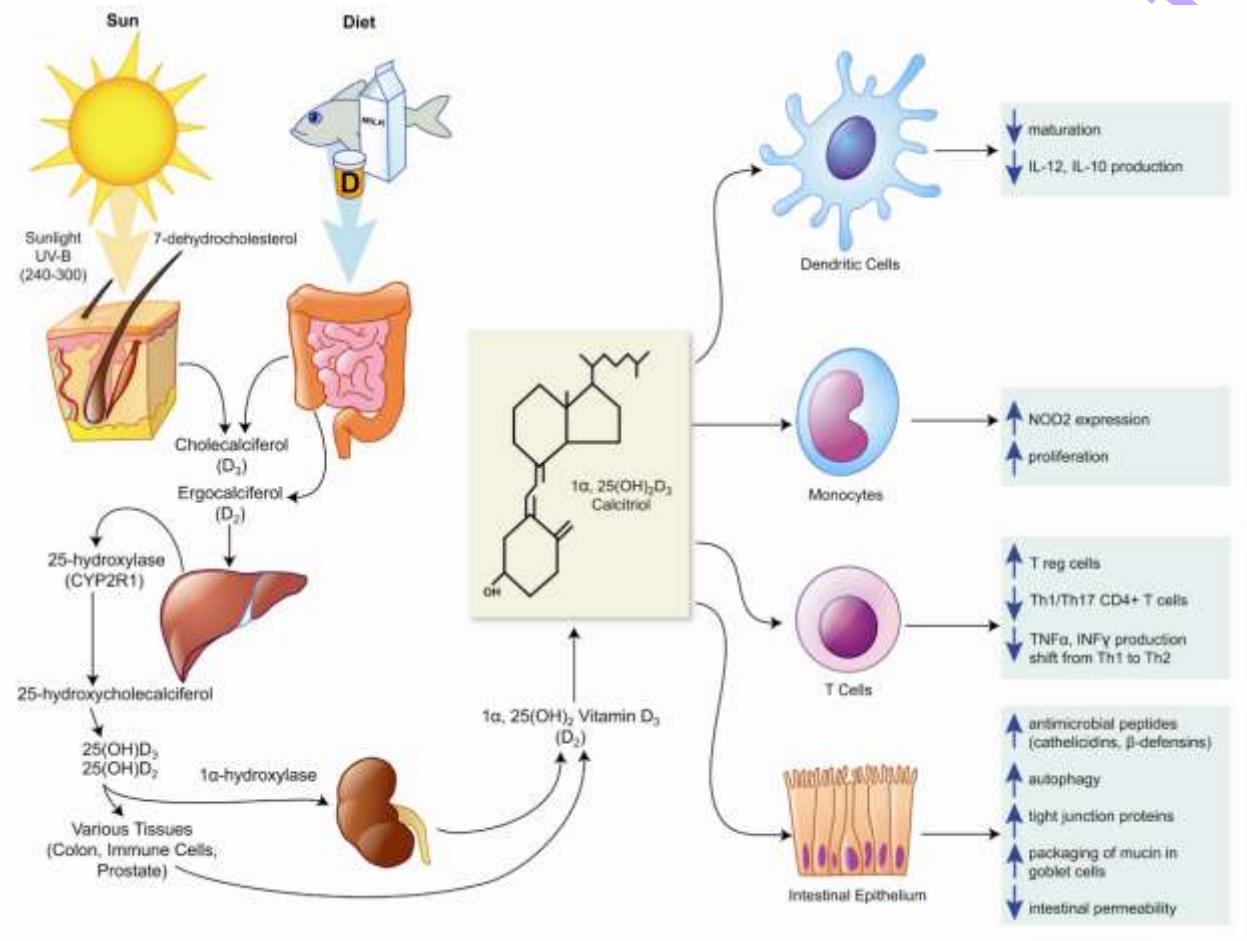
Accepted Manuscript

Yang (2013) [90]	18 adult CD	5000 IU**	24 weeks	16 ± 10	45 ± 19
Pappa (2012) [122]	61 children IBD	A: D2 2000 IU/day B: D3 2000 IU/day C: 50,000 IU /week	6 weeks	A: 16.1 ± 1.0 B: 14.7 ± 0.8 C: 15.3 ± 1.0 **	A: 25.7 ± 2.2 B: 31.5 ± 1.9 C: 40.8 ± 2.6

* Calculated from original data, reported as nmol/L using the formula 1 ng/ml = 2.49 nmol/L.
** Mean ± SE.
*** Therapy initiated at 1000 IU/day and increased every 2 weeks to a maximum of 5000 IU/day or until serum vitamin D >40 ng/ml; 14/18 patients required 5000 IU/day.
Abbreviations used: IBD – inflammatory bowel diseases, CD – Crohn´s disease, IU – international units, g – gram, Ca- calcium

Accepted Manuscript

Figure 1



Accepted