**Vitamin D treatment for connective tissue diseases: hope beyond the hype?**

John A Reynolds1,2, Ian N Bruce1,2

1. Centre for Musculoskeletal Research, Institute of Inflammation and Repair, Manchester Academic Health Science Centre, University of Manchester, Manchester, UK
2. NIHR Manchester Musculoskeletal Biomedical Research Unit, Central Manchester University Hospitals Foundation Trust, Manchester Academic Health Science Centre, Manchester, UK.

Short title: Vitamin D in CTDs

Corresponding Author:

Ian N Bruce

Arthritis Research UK Centre for Epidemiology  
Centre for Musculoskeletal Research  
Institute of Inflammation and Repair  
Stopford Building, University of Manchester  
Oxford Road  
Manchester  
M13 9PT

+44 (0)161 275 1670

Email: ian.bruce@manchester.ac.uk

The authors declare they have no conflict of interest

**Word count = 4191**

**Abstract**

The prevalence of vitamin D deficiency is increased amongst patients with connective tissue diseases (CTDs). The active form of vitamin D (calcitriol) is a potent regulator of the immune system and may suppress inflammatory responses. This has led to claims that vitamin D may be a safe treatment, or treatment adjunct, to reduce systemic inflammation in this patient population. It is important, however, that there is sufficient evidence from robust clinical trials to support these novel uses for vitamin D. In this review we examine the potential role of vitamin D as a treatment adjunct for CTDs. We will discuss how vitamin D may modulate the immune response and review the current evidence for using vitamin D to treat CTDs and their associated co-morbidities. We conclude that whilst there is much excitement about vitamin D in this context, further well designed trials are needed to demonstrate its efficacy in the treatment of patients with CTDs.

**Keywords:** vitamin D, systemic lupus erythematosus, connective tissue disease, inflammation, cardiovascular disease

**Introduction**

Vitamin D is a steroid hormone important for calcium homeostasis and the maintenance of bone health [1]. In humans approximately 80% of vitamin D is obtained by the photoconversion of 7-dehydroxycolesterol (7-DHC) into pre-vitamin D3 by ultraviolet light in the skin [2,3]. 7-DHC subsequently undergoes non-enzymatic transformation into vitamin D3 (cholecalciferol). A second form, vitamin D2 (ergocalciferol), is obtained principally from the diet [4]. Both ergocalciferol and cholecalciferol are relatively biologically inert. Activation occurs in a 2 stage process; initially 25-α hydroxylation within hepatic microsomes, resulting in the formation of 25-hydroxyvitamin D (or 25(OH)D) [5]. Whilst 25(OH)D has some activity at the vitamin D receptor (VDR), the 1-α hydroxylated form (1,25(OH)2D or calcitriol) has approximately 10x greater potency. Although 1-α hydroxylase (CYP27B1) was first identified within the mitochondria of the kidney, other tissues contain this crucial enzyme [6].

Recently there has been interest in other roles of vitamin D, particularly in relation to inflammatory diseases [4]. The VDR has been identified in a number of tissues including the skin and vasculature [7]. In the general population, large prospective observational studies have identified associations between vitamin D deficiency and a number of chronic illnesses [8].

The definitions of vitamin D deficiency and sufficiency remain controversial even within the general population. Whilst a threshold of 20ng/ml (50nmol/l) has been proposed, based on the observation that serum PTH begins to rise below 20ng/ml [9], demineralised osteoid is identified at post-mortem with concentrations below 30ng/ml (75mmol/l) [10]. A consensus opinion in 2005 suggests a target of 28-32ng/ml (70-80nmol/l) [11]. A number of high risk groups have been reported including pregnant women, older adults and ethnic groups [12]. It is not known whether these different groups require different target vitamin D concentrations.

Low vitamin D has been reported in patients with connective tissue diseases (CTDs) including systemic lupus erythematosus (SLE) [13], primary Sjogren’s syndrome (SS) [14] and idiopathic inflammatory myopathy (IIM) [15]. Whilst the prevalence of vitamin D deficiency is well-recognised, its role in the development, progression and clinical manifestations of CTDs is not clear. Many rheumatologists remain uncertain about which patients should be tested, how deficiency should be treated, and what benefits to expect from such interventions.

This review will assess the evidence for the use of vitamin D to treat CTD manifestations beyond its effects on bone health. We will focus primarily on data from observational and interventional studies in SLE although other CTDs will also be considered.

**Prevalence of vitamin D deficiency in SLE**

Compared to healthy controls, patients with SLE have an increased risk of vitamin D deficiency. On reviewing studies which included a healthy control comparator group, lower vitamin D levels were reported in SLE patients in 12/14 (86%) such studies (see supplementary table available at Rheumatology online). Conversely, Mandal *et. al.* (2014) found no difference in 25(OH)D levels between SLE patients and healthy controls although there was marked vitamin D deficiency amongst the control group. Furthermore, 39% of the patients SLE were taking steroids and calcium/vitamin D supplements which may have masked any differences [16]. Similarly, Stockton *et. al.* (2012) also found no difference in 25(OH)D between SLE patients and controls, which may be explained by 13/24 (54%) patients taking vitamin D supplements compared to 2/21 (10%) controls [17]. Both of these studies may therefore have underestimated the prevalence of vitamin D deficiency in the patient populations.

**Causes of vitamin D deficiency in SLE**

The true relationship between vitamin D and inflammation remains to be ascertained. CTDs are chronic, often debilitating diseases with high levels of morbidity. In healthy subjects, reduced physical activity and reduced sun exposure (but not dietary intake) are important determinants of vitamin D status [18]. Vitamin D deficiency in the context of chronic illness may simply reflect reduced outdoor activity, and thus UV exposure in these patients (reverse causation). This is relevant in SLE due to the photosensitive nature of the disease and the recommendations of sunlight avoidance and high SPF sunblock use [19].

A further explanation for the association between vitamin D deficiency and autoimmune disease is that 25(OH)D may act as a negative acute-phase reactant. In a meta-analysis, low serum 25(OH)D was reported following an acute event (including orthopaedic surgery and acute pancreatitis) in 6/8 studies often in association with a fall in serum albumin and rise in C-reactive protein [20]. It is proposed that this fall occurs due to reduced levels of the vitamin D binding protein (DBP) [21].

Finally, it is plausible that vitamin D deficiency may drive the development of autoimmune disease and potentiate the inflammatory response. An early study by Kamen *et. al.* (2005) identified lower serum 25(OH)D observed in recently diagnosed SLE patients compared to healthy controls and suggested that vitamin D deficiency may be a risk factor for the development of SLE. In this study however, the patients already had SLE, a condition which often has prolonged delays in diagnosis. Furthermore, the difference in vitamin D levels was only statistically significant for Caucasian patients (62% of the study cohort). In terms of leading to the development of autoimmunity, a small cross-sectional study of European Americans found significantly increased vitamin D deficiency in ANA-positive healthy controls compared to ANA-negative [22]. In a linkage-analysis study, admission to hospital for vitamin D deficiency (including osteomalacia or rickets) was associated with increased future risk of developing a number of immune-mediated diseases including RA, SLE and SS [23]. Other large prospective studies of women have not identified any association between vitamin D intake (as assessed by dietary questionnaire) and risk of developing SLE [24,25]. These observations may however reflect the inadequacy of using dietary questionnaires to estimate vitamin D status, particularly given the importance of cutaneous synthesis of vitamin D.

There is evidence that vitamin D deficiency may have a role once autoimmunity has developed. In a study by Zold *et. al.* (2008), patients who progressed from an undifferentiated CTD (UCTD) to a clearly defined CTD had significantly lower vitamin D levels than those that did not progress [26].

**How might vitamin D deficiency lead to the development of SLE?**

*Polymorphisms in the vitamin D pathway*

The vitamin D receptor (VDR) genotype may provide a link between low serum 25(OH)D levels and SLE. The Fokl polymorphism (rs2228570) is notable as the FF genotype was associated with low 25(OH)D levels in a small genetic study [27] and an increased risk of SLE in a separate larger study [28]. A further VDR polymorphism Bsml (rs1544410) has also been associated with an increased risk of SLE in Asian subjects, although it is not clear whether this is independent of serum 25(OH)D levels [28]. If vitamin D status is partly dependent on genetic variation then a fixed definition of deficiency across populations may not be appropriate. The normal range for 25(OH)D may need to be redefined on the basis of VDR genotype.

*Vitamin D and immunomodulation*

Vitamin D has broad immunomodulatory effects across both the innate and adaptive immune systems (figure 1). Of particular relevance to SLE, calcitriol reduces anti-dsDNA antibody production by inhibiting B cell proliferation, reducing differentiation into plasma cells and by B cell apoptosis [29,30]. A fall in anti-dsDNA has also been demonstrated *in vivo* after 4 weeks of high-dose cholecalciferol [31]. This effect was not due to changes in memory B cells alone. Increases in naïve and regulatory T cells, and a decrease in Th1 and Th17 cells were also observed. Furthermore, in terms of T cells, *in vitro* studies have also shown that the VDR is up-regulated following T cell activation and that calcitriol can polarise cells towards the Th2 phenotype [32]. Similarly, in a clinical study high dose cholecalciferol resulted in a reduction in the IFNγ/IL-4 ratio, representing a Th1→Th2 shift [33]. In healthy subjects, high-dose vitamin D also increases the number of FoxP3+ regulatory T cells [34].

In the innate immune system, dendritic cells (DCs) and monocytes express both the VDR and CYP27B1. The expression of CYP27B1 in monocytes, and thus local calcitriol concentration, is under regulatory control by other immune cells. The effect of calcitriol on innate immune cells is predominantly immunosuppressive with reduced monocyte differentiation and DC maturation, down-regulation of MHCII and CD80/86, leading to reduced T cell activation [35-38]. This has relevance for SLE as calcitriol attenuates monocyte expression of MHCII, CD40 and CD86 in response to lupus serum [39]. In a study of patients with type 1 diabetes mellitus, vitamin D supplementation resulted in reduced differentiation of monocytes into DCs, and the expansion of an intermediate cell type (IC), phenotypically similar to tolerogenic dendritic cells [40]. In addition, Toll-Like Receptor 9 (TLR9) senses hypomethylated DNA present in immune complexes and other sources, promoting an inflammatory response [41]. Vitamin D down-regulates TLR9 expression in healthy human monocytes resulting in reduced IL6 production in response to TLR9 stimulation [42].

Much less is known about the effects of vitamin D on neutrophil function. In animal models, calcitriol reduces neutrophil recruitment, possibly via an effect on interleukin-8 [43]. Vitamin D may also have direct effects on neutrophil function as neutrophils express VDR mRNA and show differential gene expression in response to 1,25(OH)2D3 [44]. The relevance of these changes remains to be examined in the clinical setting.

**Low vitamin D and lupus disease activity**

The relationship between 25(OH)D and lupus disease activity remains unclear. Whilst some studies have demonstrated an association [16,27,45-49], others have not [22,50-53] (see supplementary table for details). This discrepancy cannot be clearly explained but there are many potential confounding factors. As an example, the largest of these studies was conducted by Amital *et. al.* (2010) and comprised 378 patients from several European and Israeli cohorts [49]. A modest association was seen between vitamin D level at a single time point and disease activity (r=-0.12, p=0.018). In this study, disease activity was not defined using a standardised scoring system and no attempt was made to adjust for important cofounders such as use of corticosteroids, immunosuppressant drugs, vitamin D supplements or body mass index. It is therefore difficult to conclude any causative association from this observation. Furthermore, even if confirmed, the strength of the correlation suggests that, at best, vitamin D only accounts for around 1.4% of the variance in disease activity. Two other studies have also shown an association between lower vitamin D levels and lupus flares [54,55]. These observational studies do not prove causation, as low levels may still be a consequence of systemic inflammation, and the acute phase response, as described previously.

**Interventional studies of vitamin D in SLE**

Only a small number of interventional studies have investigated the effect of vitamin D on disease activity in SLE, the majority of which have been inconclusive. The largest observational study of 763 SLE patients explored the relationship between changes in vitamin D and disease activity. Whilst it was not a true interventional trial, deficient patients were treated with 50,000IU ergocalciferol (plus 200IU cholecalciferol) as per local guidelines. Although this could be considered a “high dose” regimen, the mean increase in serum 25(OH)D was not reported. The authors identified small changes in disease activity (reduction in SELENA-SLEDAI score of 0.22 [95% CI -0.41, -0.02] for a 20ng/ml increase in vitamin D) and urinary protein-creatinine ratio, (4% [2%, 5%] decrease in PCR for a 20ng/ml increase in vitamin D) in response to vitamin D therapy. The association between change in serum 25(OH)D and disease activity was only apparent using a 2-slope model and only when 25(OH)D was <40ng/ml [56]. Such associations are also very modest and of uncertain clinical significance.

Two double-blind RCTs have also focussed on the effect of vitamin D on laboratory markers of disease activity. Abou-Raya *et. al.* (2013) reported that 2000IU/day for 12 months significantly reduced anti-dsDNA and anti-Sm titres and increased serum C4 compared to placebo [13]. In this study patients were allowed to continue baseline medication but no details of any changes in medication over the 12 month trial were described. Furthermore, only a subset of the whole trial population are reported in the paper (in the intervention group the change in SLEDAI is presented for 122/178 subjects). In contrast, a smaller study by Aranow *et. al.* (2015) failed to find any change in expression of the IFN signature in response to vitamin D. Although similar dosing regimens (2000IU/day and 4000IU/day) were used, the study was shorter (12 weeks) and again achieved incomplete vitamin D repletion (only 16/33 of treated patients at the end of the study) [57]. Recently another small high-dose crossover trial of 25,000IU/month vs. 300,000IU loading and 50,000IU/month also failed to show a change in disease activity. A limitation of this study was that only the high dose regimen increased serum 25(OH)D levels, which may have been due to the high mean baseline 25(OH)D of 31.7ng/ml [58].

A small trial randomised 40 adolescent SLE patients to 50,000IU/week cholecalciferol or placebo and identified a significant difference in SLEDAI score and ds-DNA positivity after 24 weeks [59]. The study does not clearly demonstrate that vitamin D therapy reduces disease activity, as in the treated group the SLEDAI score remained stable, buy worsened in the placebo group.

These interventional studies highlight some of the difficulties in conducting clinical trials of vitamin D including selection of the correct patient population, dose, treatment duration and the influence of potential threshold effects. Patients who were vitamin D deficient at baseline who receive adequate replacement may have greatest benefit, pointing towards the need for a personalised approach. This hypothesis does however need confirmation in well conducted clinical trials.

**Sjogren’s Syndrome (SS)**

A cross-sectional study of 107 Turkish patients with SS found lower vitamin D levels in female but not male patients compared to controls [14]. This may reflect a true gender difference, although there were only 10 (9.3%) men in the study. In contrast, Agmon-Levin *et. al.* (2012) found no difference in serum 25(OH)D between SS patients and controls, although levels were lower in patients with peripheral neuropathy (18.6ng/ml vs. 22.6ng/ml) or lymphoma (13.2ng/ml vs. 22.0ng/ml) [60]. Furthermore, there is no evidence that genetic polymorphisms in the vitamin D pathway associate with the prevalence or severity of SS [61]. No studies have investigated the effect of vitamin D treatment in SS.

**Idiopathic Inflammatory Myopathies (IIMs)**

Little is known about the role of vitamin D in the IIMs. A single study of 149 IIM patients and 290 healthy subjects, found a prevalence of vitamin D deficiency of 53-68% across the IIM subtypes compared to 21% in controls [15]. An inverse association between 25(OH)D and disease activity (assessed by physician global assessment, PGA) has been reported in juvenile DM. However the relationship was weak with a 1cm change in PGA associated with only a 1.7ng/ml change in 25(OH)D [62]. Similarly to Sjogren’s syndrome there are also no associations between VDR polymorphisms and IIM. In a Hungarian study of 89 DM and PM patients there we no differences in either VDR polymorphisms or haplotypes between the IIM patients and healthy subjects [63]. Whilst there are also no intervention studies of vitamin D in IIM, a single *in vitro* study suggests that vitamin D may be able to module the inflammatory response. The cytokine CXCL-10 is released from skeletal muscle in response to pro-inflammatory cytokines and is increased in the serum of IIM patients. VDR agonists can decrease CXCL-10 secretion by human skeletal myocytes in response to stimuli [64].

**Systemic Sclerosis (SSc)**

The potential relationship between vitamin D and skin fibrosis is complex and beyond the scope of this review. Notably, the pro-fibrotic cytokine transforming growth factor-beta 1 (TGF-β1) is increased in the serum of vitamin D deficient subjects, and the pro-fibrotic effect of TGF-β1 on epithelial fibroblasts is attenuated by exposure to 1,25(OH)2D3 [65-68]. Within fibroblasts, TGF- β1 signals via the phosphorylation of the Smad3 pathway. In animal models vitamin D analogues has been reduce skin fibrosis via activation of the Th2 pathway [69].

A number of studies have identified that vitamin D deficiency is common in SSc [70-72]. Arnson *et. al.* (2011) measured 25(OH)D in 327 European SSc patients and 141 healthy controls. In this study vitamin D levels were significantly lower in patients compared to controls (13.5[9.0] vs. 21.6[9.7]ng/ml) and were inversely associated with the severity of skin fibrosis [73]. Whilst reduced cutaneous synthesis due to skin thickening and reduced intestinal absorption have been postulated to contribute to vitamin D deficiency, analysis of vitamin D metabolites suggests that these processes actually remain intact [74]. Anti-vitamin D antibodies are prevalent in SSc although there is no evidence that they contribute to disease development or progression [75].

A small study of oral calcitriol in scleroderma spectrum disorders failed to show any benefit over placebo although only 2/27 patients had systemic sclerosis whilst 20 had morphea [76]. Cutaneous calcinosis is an important feature of limited cutaneous SSc. Given that vitamin D regulates serum calcium and mobilises calcium from bone it is important to better understand any potential role of vitamin D in SSc to ensure that vitamin D therapy does not exacerbate calcinosis.

**Vitamin D and CTD-related comorbidity**

*Cardiovascular disease*

A common observation amongst CTDs is an increased risk of cardiovascular disease (CVD). In SLE the relative risk for myocardial infarction is around 2.5-fold across all age group but up to 52-fold in younger patients [77]. Vitamin D deficiency is a proposed risk factor for the development of CVD in the general population [78]. In SLE, we have demonstrated an association between aortic stiffness and vitamin D deficiency which may be mediated via increased disease activity [79]. Ravenell *et. al* (2012) also found that lower vitamin D was associated with increased carotid plaque although in this study it was also associated with reduced disease activity [80]. The association between vitamin D and traditional CVD risk factors (e.g. hypertension, hyperlipidaemia, adiposity) is less clear. Some groups have demonstrated an association [81] whilst others have not [82]. There are currently no published interventional studies to demonstrate that vitamin D can improve cardiovascular outcomes in CTDs. However, in the general population a meta-analysis of 22 trials demonstrated a significant reduction in all-cause mortality but only a trend towards reduced cardiovascular mortality [83]. Similarly, the Women’s Health Initiative (WHI) interventional study of 36,282 post-menopausal women found that vitamin D supplementation did not affect CVD risk [84]. This study may have significantly underestimated the effect of vitamin D supplementation as the control group were allowed personal supplementation (600IU/day) which was greater than the intervention dose (400IU/day). Several ongoing trials in the general population are underway and should help to resolve this question in due course.

*Metabolic syndrome*

The metabolic syndrome is present in around 40% of SLE patients early after diagnosis [85]. Metabolic syndrome is an important risk factor for CVD and is associated with cumulative organ damage [86]. A small study of non-diabetic SLE patients found that low vitamin D was associated with increased insulin resistance and a trend towards increased metabolic syndrome, independent of BMI [87]. In the general population, increases in serum 25(OH)D were associated with significantly reduced risks of developing the metabolic syndrome over 12 months [88].

*Fatigue*

Fatigue is common in SLE (approximately 80% of patients), often in association with poor sleep, anxiety and depression [89]. In an open-label study of 80 SLE patients there was a significant correlation between change in serum 25(OH)D and change in fatigue score over a 2-year period [90]. Further encouraging findings in are seen in a randomised controlled trial of cholecalciferol in juvenile-onset SLE by Lima *et. al* (2015). In this study, there was also a significant reduction in fatigue at 24 weeks. [59].

**Which patients should be tested?**

For the rheumatologist there are few guidelines to advise which patients should be tested for vitamin D deficiency. The National Osteoporosis Society (NOS) advocates measurement in patients with bone disease (in whom vitamin D may be a treatment, or should be corrected prior to other treatments) or patients with musculoskeletal symptoms which may be due to vitamin D deficiency. However, the NOS does not recommend screening asymptomatic individuals, even if they are at a high risk of vitamin D deficiency [91]. In contrast the Endocrine Society (a US-based organisation) recommend screening a number of patient groups, including those with chronic kidney disease, hepatic failure, obesity and the African-American and Hispanic populations [92]. Therefore a SLE/CTD population may include many patients in which screening for vitamin D deficiency is appropriate on the basis of assessment of bone health alone.

**How much vitamin D is enough?**

There is currently no consensus regarding the optimum vitamin D treatment regime. The Institute of Medicine (IoM) have focussed only on dietary intake of vitamin D in the general population and recommended a conservative intake of around 600IU/day [93]. The Endocrine Society suggest higher daily intake for at risk individuals, aiming for 1,500-2000IU/day, with an upper limit of 10,000IU [92]. In the UK, the NOS recommends treatment of deficiency (25(OH)D<30nmol/l) with a loading dose of up to 300,000IU followed by a maintenance dose of around 800-2000IU/day after a period of 1 month [91]. There are no guidelines relating specifically to CTDs either in terms of target vitamin D levels or recommended regimes. Our current practise is to focus on bone protection and follow the NOS recommendations, aiming for a target concentration of >30ng/ml (75nmol/l). Any additional effects of vitamin D beyond bone protection are currently theoretical and there is no convincing evidence to encourage a move away from, or to enhance current guidelines, either in the scope of screening, the dose regimes or the ideal target vitamin D concentration.

**Vitamin D toxicity**

Vitamin D therapy is usually well-tolerated and vitamin D toxicity is rare. It has been proposed that chronic consumption of around 40,000IU/day, and serum levels in excess of 80ng/ml (200nmol/l) are required before toxicity occurs [94]. Similarly, drug-vitamin D interactions are uncommon, but hypercalcaemia may occur when vitamin D is administered concurrently with calcium supplements and thiazide diuretics [95].

**Summary and future areas of research**

There remains considerable interest in the potential use of vitamin D as an adjunct in the treatment of connective tissue diseases. Although vitamin D deficiency is common across the CTDs, in observational studies there are numerous factors which confound the association between 25(OH)D and the presence of autoimmune disease. Furthermore, vitamin D levels vary considerably over time and many studies only measure 25(OH)D at a single time-point. Mendelian randomisation studies of vitamin D pathway polymorphisms may help to identify whether vitamin D deficiency predisposes individuals to developing autoimmune disease.

Whilst experimental studies have demonstrated that 25(OH)D and 1,25(OH)2D3 are anti-inflammatory and immunoregulatory across a number of immune pathways, the results from clinical studies have been inconclusive. There is also a lack of well-designed interventional studies both in SLE and in other CTDs. Many of the studies have used relatively low doses of vitamin D and/or may have not achieved sufficient changes in 25(OH)D levels. It is likely that the optimum serum 25(OH)D level is different for individual patients and a personalised approach is likely to be needed. However, the absence of positive results may also point towards a more passive role for vitamin D in autoimmunity, with serum 25(OH)D levels acting as a negative acute phase reactant and thus reflecting the presence of systemic inflammation and general poor health rather than being causative. Well-designed trials are now needed in order to define the utility, if any, of vitamin D to influence or modify the primary disease or its comorbidities beyond its role in bone health.

**Key messages:**

* Patients with connective tissue diseases have a high prevalence of vitamin D deficiency
* High risk patients should be tested, not routinely screened, and treated to optimise bone health
* The role of vitamin D on disease activity, prognosis and non-bone comorbidities remains to be demonstrated

**Acknowledgements**

Professor Bruce is a National Institute for Health Research (NIHR) Senior Investigator and is funded by Arthritis Research UK, the NIHR Manchester Biomedical Research Unit and The NIHR Manchester Wellcome Trust Clinical Research Facility. The views expressed in this publication are those of the authors and not necessarily those of the National Health Service, the National Institute for Health Research or the Department of Health.

Figure: Summary of the effects of vitamin D on the innate and adaptive immune systems

A schematic representation of the effects of 1,25(OH)2D on the immune system derived principally from *in vitro* studies. The white arrows shows an increase in response to 1,25(OH)2D and the dark show a decrease.

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