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EDITORIAL

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In this issue you will find an update on the relationship between vitamin D and two major topics: autoimmune diseases and pain.

Vitamin D is said to have immunomodulating effects. What does that mean? Trying to provide as simple an answer as possible, I believe that vitamin D's three main immunological functions should be acknowledged: the bactericidal function, the inflammatory response attenuation function and that of promoting immune tolerance [1].

The first role, also in chronological order, is as a bactericide, as was discovered 170 years ago, when cod liver oil was used to treat tuberculosis. Although, the key mechanism was only described by the scientific community 40 years ago with the identification of vitamin D receptors in leukocytes and when it was shown that vitamin D-activating enzymes could also be found in the monocyte-macrophage line. The second function is vitamin D's ability to attenuate the inflammatory response by inhibiting proinflammatory cytokines and by stimulating those with anti-inflammatory action at the level of T lymphocytes, thus reducing possible clinical manifestations of chronic inflammatory diseases or possible damage from "friendly fire". Vitamin D's third important immunological function is how it promotes immune tolerance, by being able to slow down dendrite cell maturation and antigen presentation, as well as by inhibiting the survival, proliferation, differentiation, and antibody production of B lymphocytes.

Hence the pathophysiological rationale for understanding the risk of incurring autoimmune diseases under conditions of vitamin D deficiency also arises. In this regard, as is well known, numerous epidemiological studies have described a high prevalence of vitamin D deficiency in several autoimmune diseases, including above all, multiple sclerosis, type 1 diabetes, psoriasis, Crohn's disease and many rheumatological diseases (especially rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, systemic sclerosis, and lupus). However, these observations were unable to document any certain cause and effect correlation. Long-term longitudinal studies, preferably prospective, which explore the correlation between vitamin D status or vitamin D supplementation and the incidence of autoimmune diseases, are needed to prove this.

As you will read in this issue, it has indeed recently been observed that daily supplementation with 2000 IU of vitamin D was associated with a significant reduction in the risk of incurring autoimmune diseases [2]. This important result confirms the clinical significance of the pathophysiological assumptions, being the causal relationship. It further supports the belief that prevention of vitamin D deficiency can in effect also reduce the risk of autoimmune diseases.

The other contribution in this issue is a review of the possible role of vitamin D in pain, the main clinical expression of many diseases, especially rheumatological and oncological. Here too, the author has first of all taken care to summarise the main physiopathological assumptions that might underlie this finding, specifically identifying them in the presence of certain vitamin D receptors in the neurons of the central and peripheral pathways involved in pain detection and processing. This includes vitamin D's ability to modulate the expression of various pain-related genes, also in the presence of enzymatic activities assigned to vitamin D activation at a neuronal level, and then vitamin D's capacity to interact or interfere with neurotrophic factors or algogenic cytokines or other neuro-immunomodulators.

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Although there is a rationale for expecting a positive effect of vitamin D in pain control, as you will see, the results of the studies conducted to date are inconsistent and discordant. Nevertheless, as the author acknowledges, there are many justifications for such discrepancies. These include the still uncertain definition and determination of what vitamin D deficiency is and its variability, the many genetic polymorphisms that can affect a "functional" vitamin D deficiency as well as different individual pharmacokinetics or pharmacodynamics, personal heterogeneity, and variability in the perception of pain, which tend to compromise any accurate assessments, and which render urgent the search for the biomarkers of pain. Thus, the need for further clinical studies and translational research in this field as well is revealed.

What are your thoughts?
Happy reading!

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Vitamin D in autoimmune diseases

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INTRODUCTION

That vitamin D cannot be considered as merely a vitamin related to bone metabolism is by now an established fact. Certainly, everyone knows that exposure to sunlight improves our state of well-being. The explanation for this cannot simply be reduced to the production of endorphins by keratinocytes exposed to UV radiation [1].

There is a great deal of historical evidence for the efficacy of heliotherapy, starting with the Nobel Prize awarded to Niels Ryberg Finsen in 1903 for showing the extraordinary and rapid therapeutic efficacy that exposure to sunlight had on tubercular skin lesions [lupus vulgaris] [2]. Therefore, vitamin D goes far beyond bone metabolism alone, which is also confirmed by the observation that essentially, the vitamin D receptor (VDR) is nearly ubiquitous in our bodies being particularly well represented in extra-skeletal tissues [3]. Furthermore, this receptor has also been found in yeasts and in animals with no skeletal or dental apparatus at all, such as lampreys [4]. Among the extra-skeletal actions of vitamin D, this review will focus on that relating to the modulation of the immune response.

The VDR is expressed by diverse cells of the immune system (both innate and adaptive). However, many of these cells (especially macrophages and dendritic cells) possess the full enzyme apparatus required to transform vitamin D into its active form, which will then act on the same cell (autocrine activity) or on neighbouring cells (paracrine activity) [5].

VITAMIN D AND IMMUNE CELLS

In recent years, the effect of vitamin D on immune cells has been studied a great deal. This research is summarised in Table I. Monocytes/macrophages play a key role in protecting against infection by producing proinflammatory cytokines. The binding of pathogenic components (bacterial, viral, or fungal) to toll-like receptors expressed on the surface of monocytes and macrophages induces the overexpression of VDR and of the CYP27B1 cytochrome, which is essential for the activation of vitamin D inside the cell. The intracellular binding of activated vitamin D [$1,25(OH)_2D$] with the VDR forms a heterodimer, which, binding to the DNA, induces the production of cathelicidin and β-defensins. Once these antibacterial peptides have been released at the extracellular level, they act by directly destroying the cell membranes of bacteria and viruses or by activating other innate defence mechanisms such as autophagy [6]. Dendritic cells act as antigen-presenting cells to T cells, thus triggering the adaptive immune response.

In the presence of the active form of vitamin D there is a downregulation of the Major Histocompatibility Complex Class II (MHC Class II) molecules and costimulatory molecules (e.g., CD40, CD80 and CD86), expressed on dendritic cells, resulting in less T-cell activation. This is also associated with an inhibiting effect on the production of the IL-12 and IL-23 proinflammatory cytokines (and thus also on IL-17] as well as stimulating the production of IL-10, which has an anti-inflammatory effect [6]. Once the T-cells are activated by antigen-presenting dendritic cells, they induce an antigen-specific immune response. Even T lymphocytes express both VDR and CYP27B1. It is interesting to note, however, that there are low levels of VDR in naïve T lymphocytes, with values that progressively increase, after their cellular activation. The effect of activated vitamin D [$1,25(OH)_2D$] is to:

- reduce Th1 differentiation
- reduce the production of inflammatory cytokines (IL-2, IFNγ and TNF-α)
- reduce Th17 differentiation
- promote Th2 differentiation
- promote the secretion of anti-inflammatory cytokines (IL-4, IL-5 and IL-10)
- promote the differentiation of regulatory T lymphocytes.

All these actions ensure careful modulation of the immune response and the prevention of its exaggerated activation [6,7], which is always possible, and which is the basis of autoimmune diseases.

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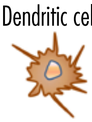
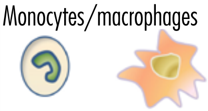
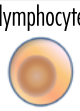
B lymphocytes in the immune system are the key players in the production of autoantibodies. These cells also express VDR and CYP27B1. In their case as well, vitamin D activation appears to have a predominantly regulatory role, which is achieved by both direct and indirect mechanisms.
The indirect mechanism occurs through the suppression of B-cell differentiation, proliferation, and antibody production by $1,25(OH)_2D$-treated T helper cells. Whereas the direct mechanism occurs through vitamin D's effect of suppressing B-cell differentiation and/or their maturation to memory cells and plasma cells [6].
If we consider the general action of vitamin D (in its active form) on the immune system (Table I) it becomes quite clear how the innate destructive capacity of the various pathogens is stimulated, whilst at the same time there is also a consensual modulation of the antigen-specific adaptive response. In fact, the role of the Th1 response is to amplify the inflammatory response which must in turn be somehow controlled by the Th2 response. It appears that vitamin D acts in favour of this type of "control". Interestingly, though the action of $1,25(OH)_2D$ is always inhibitory to lymphocyte cells, the degrees of inhibition are very different. There appears to be a marked inhibition of the cells that support and amplify Th1, Th17 and B cells, whilst the inhibitory effect seems be much milder on the cells that regulate the immune response (Th2 and T-reg cells). Hence, the end result, in the presence of adequate vitamin D levels, would be a "relative stimulation" of these latter cells resulting in an immunomodulatory action [7] (Fig. 1).

VITAMIN D AND AUTOIMMUNE DISEASES

Results from numerous epidemiological studies leave no doubt as to the high prevalence of vitamin D deficiency in subjects with autoimmune rheumatic diseases! Patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA), ankylosing spondylitis (AS), systemic sclerosis (SS) and systemic lupus erythematosus (SLE) have circulating levels of 25-hydroxy-vitamin D [25(OH)D] at least 8-10 ng/mL lower than healthy controls [8].
The CARMA study [9] compared the vitamin D status of 2,234 patients affected by RA, PsA and AS with that of 667 healthy subjects with similar ages and found vitamin D deficiency [serum 25(OH)D < 20 ng/mL] in 40-41% of the patients compared to 27% of the healthy subjects ($p < 0.001$).

TABLE I.
Immunological effects (autocrine and paracrine) relating to the activation of vitamin D at the level of immune cells.

TYPE OF CELL	ACTION ON THE CELL	ACTION ON RELATED CYTOKINES
Dendritic cell 	– Inhibition of maturation – Inhibition of antigen presentation	– Inhibition of production: IL-12, IL-23, IL-17 – Stimulation of production: IL-10
Monocytes/macrophages 	– Increased differentiation – Increased bactericidal activity – Increased production of bactericidal substances	
T lymphocytes 	– Th1 response inhibition – Th17 differentiation inhibition – Th2 response induction – T-regs differentiation induction	– Inhibition of production: IL-2, IFNγ, TNF-α, IL-17 – Stimulation of production: IL-4, IL-5 and IL-10
B lymphocytes 	– Inhibition of proliferation – Inhibition of differentiation into plasma cells – Inhibition of antibody production	

Moreover, a correlation also emerged from the statistical analysis (albeit at the limits of significance) between vitamin D deficiency and the RA severity indicator (higher risk of ACPA positivity in RA patients with an OR = 1.45; 95% confidence interval (95% CI) 0.99-2.12 and $p = 0.056$) and with functional impairment from AS (higher risk of disease-related functional impairment with an OR = 1.08; 95% CI 0.99-1.17; $p = 0.07$) [9]. Despite the data available, it is still difficult to establish a cause-and-effect relationship between vitamin D deficiency and autoimmune diseases with any degree of certainty. In some animal models of autoimmune diseases, vitamin D was found to slow down their development and/or progression [10]. Instead, results from some observational studies in humans appear to be contradictory, whilst those concerning vitamin D supplementation of subjects with confirmed autoimmune diseases have been generally disappointing [10].
Hence at present, there is insufficient evidence supporting the possible efficacy of vitamin D supplementation in preventing the onset of autoimmune diseases. Long-term longitudinal studies performed in the general population would be required. Finally, this type of study has become available, and the results appear to be truly encouraging. The recently published VITAL study set out to investigate if vitamin D (whether or not associated with long-chain omega-3 fatty acids) can reduce the risk of autoimmune diseases.[10] This was a randomised, double-blind, placebo-controlled clinical trial conducted in the United States that involved 25,871 subjects (12,786 men ≥ 50 years and 13,085 women ≥ 55 years) who were followed for an average of more than 5 years. The randomised subjects were to take 2,000 IU of vitamin D (or placebo) and omega-3 fatty acids (1,000 mg/day) or placebo daily. By the express admission of the researchers who conducted it, the aim of the study was not to analyse the effects of vitamin D supplementation on a cohort of vitamin D-deficient subjects, but rather on a representative sample of elderly Americans in the general population. Then, the fact that subjects with a history of chronic kidney or liver disease, with hypercalcaemia, malignant tumours, cardiovascular disease, or other serious illnesses were excluded from enrolment, meant that only substantially healthy subjects were selected. Hence, it was no surprise that the number of subjects who developed

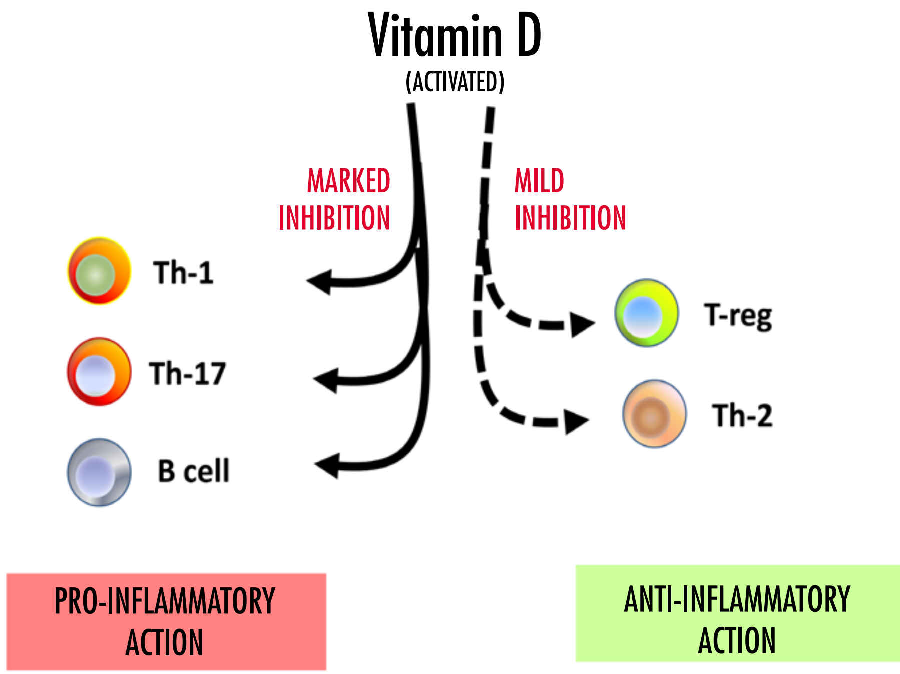


FIGURE 1.
Effect of vitamin D on adaptive immunity. Since the inhibitory effect is much more pronounced on the pro-inflammatory side, the final effect will be one of response control and modulation.

the autoimmune diseases considered in the course of the study was in any case small in absolute numbers (278 cases, which in practice, represents an incidence of new cases of just over 1%, over the five years of observation). The diseases considered were rheumatoid arthritis, polymyalgia rheumatica, autoimmune thyroid diseases, psoriasis, and inflammatory bowel diseases. However, there was in any case a field where clinicians could write in all other new-onset autoimmune diseases. The results showed that daily supplementation with vitamin D (for 5 years) with or without omega-3 fatty acids ensures a statistically significant 22% reduction in the occurrence of autoimmune diseases (with confirmed diagnosis).
The large amount of data made available by this study has permitted us to propose some very interesting considerations:

- compared to the placebo reference arm (placebo with vitamin D and placebo with omega 3 fatty acids) the significant risk reduction (again considering only cases with confirmed diagnoses) that emerged was only among those who had received vitamin D and omega 3 fatty acids together (OR = 0.69 with 95% CI 0.49-0.96: p = 0.03), or who had received vitamin D alone (OR = 0.68 with CI 95% 0.48-0.94: p = 0.02), but was not among those who had received omega-3 fatty acids alone (OR = 0.74 with CI 95% 0.54-1.03, p = 0.07 not significant);
- since autoimmune diseases develop slowly over time [11], an additional analysis was also included in the study, which excluded events that occurred during the first two years and considered only the last three years of the study. Even in this case, the group treated with vitamin D had a 39% reduced incidence of confirmed autoimmune disease compared to placebo (p = 0.005). Whereas the group treated with omega-3 fatty acid showed only a 10% reduction in new cases of confirmed autoimmune disease compared to placebo, which did not even achieve statistical significance (p = 0.54).

CONCLUSIONS

My conclusions are very much in line with those of the authors of this extraordinary study that I just presented here and with whom I agree completely. It is well known that autoimmune diseases are a group of heterogeneous disorders that often present similar pathogenetic mechanisms, accompanied by severe consequences in terms of both morbidity and mortality. Having for the first time clearly demonstrated how in a population of essentially healthy elderly subjects, constant daily supplementation of 2,000 IU of vitamin D (alone or in combination with omega-3 fatty acids) is able to reduce the incidence of autoimmune disease with more pronounced effects after two years, is of no small import. After all, this supplementation bears no risk of toxicity and is well-tolerated in the face of the total lack of treatments that are currently effective in reducing the incidence of autoimmune diseases.
I would hope that there shall soon be studies of similar quality investigating this preventive opportunity among younger subjects and perhaps among subjects at a higher risk of developing this type of disorder.

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Vitamin D and pain

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Pain, according to the recent definition of the International Association for the Study of Pain, is an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage [1]. Many pathologies, which have pain as their primary clinical expression, contribute significantly to morbidity and mortality on a global scale.

Although there is growing evidence in the literature of a possible relationship between low levels of 25-hydroxy vitamin D [25(OH)D] and different types of acute or chronic pain and how adequate vitamin D supplementation, particularly in patients with a deficiency, can lead to an improvement in pain symptoms, clinical trials conducted for this purpose have provided inconsistent or discordant results, which, from time to time, have been attributed to participant selection, outcome measures, sample size, vitamin D dosage and/or follow-up duration. Nevertheless, the potential mechanisms by which vitamin D might exert analgesic effects remain poorly understood.

Clinical research in the area of the correlation between chronic pain and vitamin D deficiency is limited. There are still very few randomised, controlled, and blinded studies. Regardless, clinical trials have shown that vitamin D is able to exert anatomical and physiological influence on the manifestation of pain, thus playing a positive role in the aetiopathogenesis and maintenance of chronic pain states and associated comorbidities. Manifestations of pain associated with immunological, hormonal, and neuronal changes are potentially influenced by vitamin D levels. Indeed, low vitamin D levels have been found in patients with various pain states such as headache, abdominal pain, knee pain, low back pain, persistent musculoskeletal pain, costochondritis chest pain, *"failed back syndrome"* and fibromyalgia.

EXPERIMENTAL FINDINGS

The interaction between vitamin D and its VDR receptor appears to play a role in improving pain symptoms through the modulation of key genes associated with pain. Some of these pain genes are common to both superficial and visceral nociception, e.g., TRPV1, the toll-like receptor, trophic factors such as NGF, GDNF and EGFR (Table I). Furthermore, the hypothesis that vitamin D may influence pain signalling pathways is biologically plausible because the gene expression of vitamin D and/or of its VDR receptor, has been demonstrated in the skin (transduction of pain signalling), in the dorsal root ganglion (DRG) neurons (conduction), in the spinal cord (transmission/modulation) and in the brain (pain perception) (Figure 1). The expression of the Vitamin D receptor has been reported in peripheral and central neurons involved in pain sensing and processing. Expression of transcription for the nuclear vitamin D receptor and/or enzymes regulating the active form of vitamin D levels have been demonstrated in the nerve fibres of DRG neurons terminating in the skin, in neurons of the spinal cord and of the brain. The level of VDR transcription in DRG neurons is higher than in other regions of the nervous system. Vitamin D activity is determined by two enzymes, CYP27B1, which activates vitamin D in the kidney and CYP24A1, which inactivates active vitamin D. These two enzymes, together with VDR, are also expressed in nociceptor neurons and in the brain [2].

TABLE I.
Role of trophic factors, influenced by vitamin D, in the pathogenesis of pain.

Trophic factor	Role
Nerve Growth Factor (NGF)	Development of nociceptor neurons and pain processing
Glial cell line-derived neurotrophic factor (GDNF)	Survival and activity of large cutaneous sensory and proprioceptive neurons
Epidermal Growth Factor (EGFR)	Hub or main relay in pain processing and detection

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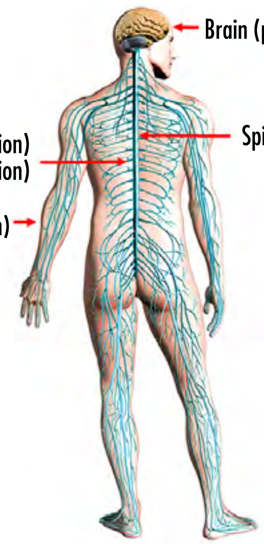

FIGURE 1.
Action of vitamin D on pain transmission pathways.

Vitamin D and VDR play a role in pain signal transduction. Vitamin D interacts with the nerve endings of the nociceptive neurons in the skin to directly detect painful proinflammatory stimuli and to control the activity of the TRPV1 channel in T lymphocytes. The VDR could play a role in modulating the expression of pain genes, e.g., those involved in the development of neurons and Schwann cells, and ion channels expressed in nociceptive neurons that innervate the skin as well. Alterations in the expression or function of vitamin D regulating enzymes, VDR expression, VDR targets on the skin and/or on sensory neurons or associated glial cells could probably have an impact on chronic pain conditions such as neuropathic pain and painful diabetic neuropathy [3].

The interaction between vitamin D and Nerve Growth Factor (NGF) influences nociceptive signal processing. Vitamin D increases the expression of NGF in the DRG neurons innervating the skin in rats, as it does in hippocampal neurons. NGF is a neurotrophic factor necessary for the development and maturation of nociceptors. Under pathological conditions, it appears that NGF levels increase in response to inflammation. In addition, NGF stimulates the release of calcitonin gene-related peptide (CGRP) from peripheral DRG neuron endings. It is believed that CGRP promotes and maintains nociceptive neurons sensitised, which also implies its role in chronic pain. Sensitisation is also enhanced by NGF-facilitated augmented insertion of TRPV1, an ion channel involved in the response to thermal stimulus in the cell membrane. Furthermore, the transcription level of various sodium channel isoforms (e.g., Nav1.6, Nav1.7, Nav1.8 and Nav1.9) is modulated by NGF and ultimately results in increased sodium current density and sensitivity to nociception, mainly by way of Nav1.8. In addition, the development of hyperalgesia during inflammation is believed to stem from an NGF-promoted increase in Nav1.7 expression. It follows that NGF is crucial for the development of nociceptor neurons and for pain processing. Yet, it is still unclear whether this is a direct effect of vitamin D on NGF or if this is an indirect outcome achieved by way of extranuclear or nuclear signalling pathways [4].

Another neurotrophic factor, called Glial cell line-derived neurotrophic factor (GDNF), expressed in a small population of DRG neurons, is implicated in promoting the survival and activity of large cutaneous sensory and proprioceptive neurons. GDNF plays a central role in pain transmission. Recent studies have shown that GDNF and its C-Ret receptor are directly regulated by vitamin D. It could be hypothesised that both vitamin D and its receptor might play a role in sodium channel-mediated neuropathic pain through modulation of GDNF expression. However, experimental verification of this is required [5].

The Epidermal Growth Factor Receptor (EGFR) and its effectors have recently been identified as novel signalling pathways involved in pain processing. It is known that their expression is also regulated by vitamin D. EGFR is widely expressed in cells of the body including in epithelial cells, neurons involved in pain transmission and skin keratinocytes, the latter being a primary source of vitamin D for the body. Though dysregulation of EGFR signalling is believed to underlie the pathogenesis of several cancers, there is also evidence of its role in other pain-causing pathologies as well as in the mechanisms underlying pain detection and processing. EGFR is a key player in pain processing and detection. Since it is already known that EGFR acts as a significant signal hub and relay from a variety of stimuli, its new role in pain signal processing has provided added value to its proposed designation as a "primary hub or relay" for cell signalling. Thus, the inhibition by vitamin D of this important signalling hub, comprising EGFR, could explain its analgesic effects. Actually, several studies have suggested that vitamin D inhibits EGFR gene expression either directly or indirectly [6].

CLINICAL TRIALS (Table II)

Warner et al. evaluated the effect of vitamin D treatment in patients with diffuse musculoskeletal pain and osteoarthritis (controls). Patients with 25-hydroxyvitamin D levels ≥ 20 ng/mL were randomised to receive placebo or 50,000 IU of ergocalciferol once a week for 3 months. Vitamin D treatment had no effect on pain compared to baseline [Visual Analog Scale (VAS) $p = 0.73$; Functional Pain Score (FPS) $p = 0.18$] or at 3 months compared to placebo (VAS $p = 0.12$; FPS $p = 0.05$, in favour of placebo). The authors concluded that low levels of vitamin D are not associated with widespread musculoskeletal pain and vitamin D treatment does not reduce pain in patients with widespread pain who have low levels of vitamin D [7].

Schreuder et al. studied the effect of high-dose vitamin D_3 on persistent, non-specific musculoskeletal disorders in vitamin D-deficient non-Western immigrants and assessed the correlation between pain type and benefit from the treatment. Patients were randomised to placebo or vitamin D (150,000 IU of oral vitamin D_3). At week 6, patients in the original vitamin D group were randomised a second time to receive vitamin D (again) or to switch to placebo, whilst all the patients in the original placebo group were switched to vitamin D. Patients in the vitamin D group were significantly more likely than the placebo group to report pain relief

TABLE II.
Clinical studies on the use of vitamin D in different painful disorders.

Clinical trial	Condition	Efficacy on pain
Warner AE, 2008 [7]	Musculoskeletal pain	-
Schreuder F, 2012 [8]	Musculoskeletal pain	+
McAlindon T, 2013 [9]	Osteoarthritis of the knee	-
Sanghi D, 2013 [10]	Osteoarthritis of the knee	+
Rastelli AL, 2011 [11]	Breast cancer	+
Wepner F, 2014 [12]	Fibromyalgia	+
Sakalli H, 2012 [13]	Pain in the elderly	+
Gendelman O, 2015 [14]	Musculoskeletal pain	+
Jin X, 2016 [15]	Osteoarthritis	-
Wu Z, 2018 [16]	Pain in the general population	-
Frankling MH, 2021 [17]	Pain in cancer patients	+

six weeks after treatment (34.9% *vs* 19.5%, p = 0.04) and a better ability to climb stairs (21.0% *vs* 8.4%, p = .008). Therefore, 6 weeks after a high dose of vitamin D, a small positive effect was found on persistent non-specific musculoskeletal pain [8].

McAlindon et al., in a study to determine whether vitamin D supplementation reduces symptoms and structural progression of osteoarthritis of the knee, randomised participants to receive placebo or 2,000 IU/day of oral cholecalciferol, with a dose increase to raise serum levels to more than 36 ng/mL. Knee pain decreased in both groups by an average of -2.31 [with 95% confidence interval (CI 95%), -3.24 to -1.38] in the treatment group and by -1.46 (CI 95%, -2.33 to -0.60) in the placebo group, with no significant differences at any time. The percentage of cartilage volume decreased by the same amount in both groups (mean, -4.30; 95% CI, -5.48 to -3.12 vs mean, -4.25; 95% CI, -6.1 2 to -2.39) (p = 0.96). There were no differences in any of the secondary clinical endpoints. In this study, vitamin D supplementation for two years at a dose sufficient to elevate plasma 25-hydroxyvitamin D levels to more than 36 ng/mL, compared to placebo, did not reduce knee pain or cartilage volume loss in patients with symptomatic knee osteoarthritis [9].

Sanghi et al. conducted a study to investigate whether vitamin D treatment could reduce knee pain, improve function, and change the levels of relevant biochemical markers in patients with knee osteoarthritis and vitamin D deficiency. At 12 months, knee pain had decreased in the vitamin D group by an average of -0.26 (95% CI, -2.82 to -1.43) on VAS and -0.55 (95% CI, -0.07 to 1.02) on WOMAC, whilst in the placebo group, it increased by an average of 0.1 3 (95% CI, -0.03 to 0.29) on VAS and 1.16 (95% CI, 0.82 to 1.49) on WOMAC (effect size = 0.37 and 0.78). In the same manner, knee function improved in the vitamin D group by an average of -1.36 (95% CI, -1.87 to -0.85) compared to the placebo group which had an average of 0.69 (95% CI, -0.03 to 1.41; effect size = 0.06). There were significant biochemical changes in serum total calcium, 25(OH)D and alkaline phosphatase. The study results suggest that there is a small, but statistically significant, clinical benefit to vitamin D treatment in patients with knee osteoarthritis [10].

Rastelli et al. conducted a randomised, double-blind, placebo-controlled phase II study to determine whether vitamin D supplementation or high-dose supplementation (HDD) in women receiving anastrozole as adjuvant therapy for breast cancer improves aromatase inhibitor-induced musculoskeletal symptoms (AIMSS) and bone loss. Patients with early-stage breast cancer and AIMSS were stratified according to their baseline level of 25-hydroxyvitamin D [25(OH)D]. Group A (20-29 ng/mL) received HDD capsules 50,000 IU weekly for 8 weeks and then monthly for 4 months or placebo. Group B (10-19 ng/mL) received HDD for 16 weeks and then monthly for 2 months or placebo. At 2 months, all pain scale scores were improved in the HDD group compared to the placebo group. Femoral neck BMD decreased in the placebo group but was unchanged in the HDD group (p = 0.06). The study showed that weekly HDD improves AIMSS and can have a positive effect on bone density. The authors suggest that vitamin D supplementation strategies for breast cancer patients on aromatase inhibitor therapy should be investigated further [11].

Wepner et al. studied 30 women with fibromyalgia syndrome, whose serum calcifediol levels were < 32 ng/mL (80 nmol/L). The women were randomised to the treatment or control (placebo) group, with the aim of achieving serum calcifediol levels between 32 and 48 ng/mL for 20 weeks by oral supplementation with cholecalciferol. Both groups were reassessed after an additional 24 weeks without cholecalciferol supplementation. The treatment group noted a marked reduction in pain during the treatment period with a significant effect on the VAS scale scores. This was also correlated with physical role function scale scores from the *Short Form 36 Health Survey*. Optimisation of calcifediol levels in fibromyalgia syndrome had a positive effect on pain perception. The authors deemed that vitamin D therapy can be taken into consideration for patients with fibromyalgia syndrome [12].

Sakalli et al. investigated the benefits of a single dose of vitamin D, administered either orally or parenterally, on improved quality of life and functional mobility and diminished pain among elderly subjects. Community-dwelling older adult subjects over 65 years of age were included in the study. The subjects were given 300,000 IU of vitamin D, either orally or parenterally, and were assessed after 4 weeks. The subjects were divided into four groups of 30. The first group was administered IM vitamin D, the second group was administered IM placebo, the third group took vitamin D PO, and the fourth group took placebo PO. After treatment, the PTH level of the first group was reduced (p = 0.0001) and the level of vitamin D was significantly increased (P = 0.0001). In the third group, the PTH (parathormone) level was reduced (p = 0.0001), and the level of vitamin D was increased (p = 0.004) and the 24-hour calcium excretion in the urine (p = 0.015) was significantly increased. When pain, functional mobility and quality of life were assessed, the *timed up and go* test (TUG) ($p = 0.0001$) and VAS ($p = 0.0001$) scores decreased significantly in the first group, whilst the SF-36 parameters: physical function ($p = 0.0001$), physical role (0.006), physical pain ($p = 0.0001$), general health ($p = 0.007$), social function ($p = 0.05$) and mental health ($p = 0.048$) showed significant increases. In the second group, the VAS ($p = 0.001$) decreased whilst the physical role ($p = 0.009$) and emotional role ($p = 0.034$) increased significantly. In the third group, TUG ($p = 0.0001$) and VAS ($p = 0.002$) showed a decrease, whilst physical function ($p = 0.0001$) and physical role (0.001) showed significant increases. In the fourth group, the VAS ($p = 0.007$) decreased notably. The authors concluded that administration of megadoses of vitamin D increases quality of life, decreases pain and improves functional mobility in the elderly [13].

Gendelman et al. evaluated the impact of administering 4,000 IU of vitamin D, compared to placebo, on pain and serological parameters in patients with musculoskeletal pain. Eighty patients were enrolled, and the therapy was administered for three months. Parameters were assessed at three time points: before the trial, at week 6 and at week 12. The group that received vitamin D achieved a statistically significant reduction in the VAS during the study compared to the placebo group. The need for an analgesic "rescue therapy" was significantly lower in the vitamin D-treated group. TNFα (tumour necrosis factor alpha) levels decreased by 54.3% in the vitamin D-treated group and increased by 16.1% in the placebo group. PGE2 (prostaglandin E2) decreased by 39.2% in the vitamin D-treated group and increased by 16% in the placebo group. Leukotriene B4 (LTB4) levels decreased in both groups by 24% ($p < 0.05$). According to the authors, the addition of 4,000 IU of vitamin D for patients with musculoskeletal pain may lead to a more rapid decrease in consecutive VAS scores and a decrease in the levels of inflammatory and pain-related cytokines [14].

Jin et al. compared the effects of vitamin D supplementation versus placebo on pain and knee cartilage volume in patients with symptomatic osteoarthritis and low vitamin D levels. Participants were randomly assigned to receive a monthly treatment with oral vitamin D_3 (50,000 IU: n = 209) or an identical placebo (n = 204) for 2 years. The 25-hydroxyvitamin D level increased more in the vitamin D group (40.6 nmol/L) than in the placebo group (6.7 nmol/L) ($p < 0.001$) over the 2 years. There were no significant differences in the annual change in tibial cartilage volume or in the pain scores. No significant differences were found in the change in tibiofemoral cartilage defects nor in the change in tibiofemoral bone marrow lesions. These results do not support the use of vitamin D supplementation to prevent tibial cartilage loss or to improve pain in patients with knee osteoarthritis [15].

Wu et al. conducted a study with the aim of comparing the effect of monthly high-dose vitamin D supplementation on pain impact questionnaire (PIQ-6) scores and on the prescription of analgesics in the general population. Participants, aged 50-84 years, were randomly assigned to receive monthly 100,000 IU vitamin D_3 capsules (n = 2558) or placebo (n = 2550) for a median of 3.3 years. No difference was found in the mean PIQ-6 score at the end of follow-up (adjusted mean difference: 0.06; $p = 0.82$) among participants in the vitamin D group (n = 2041) or in the placebo group (n = 2014). The proportion of participants taking one or more opioids was similar in the vitamin D group (n = 559, 21.9%) compared to placebo (n = 593, 23.3%); the relative risk (RR) adjusted for age, gender and ethnicity was 0.94 ($p = 0.24$). Similar results were observed for the administration of NSAIDs (RR = 0.94; $p = 0.24$) and other non-opioid analgesics (RR = 0.98; $p = 0.34$). Focusing on participants with vitamin D deficiency (< 50 nmol/L, 24.9%), there was a lower risk of NSAID administration in the vitamin D group compared to placebo (RR = 0.87; $p = 0.009$). All other subgroup analyses were not significant. The study showed that monthly supplementation of high-dose vitamin D neither improves the mean PIQ-6 score nor reduces analgesic intake in the general population [16].

In the recent "Palliative-D" study, Frankling et al. tested the hypothesis that correction of vitamin D deficiency may reduce opioid use in cancer patients admitted to palliative care. Patients with advanced cancer and 25-hydroxyvitamin D <50 nmol/L were randomised to 4000 IU/day of vitamin D_3 or placebo for 12 weeks. The primary endpoint was the difference in long-acting opioid use (fentanyl μg/h) between the groups over 12 weeks. The treated group of patients had a significantly lower increase in opioid doses than the placebo group ($p = 0.03$). The Fatigue reduced by vitamin D, assessed using the ESAS (Edmonton Symptom Assessment Scale), was -1.1 points after 12 weeks ($p < 0.01$). According to the authors, correction of vitamin D deficiency may have positive effects on opioid use and fatigue in patients undergoing palliative treatment for cancer, but only for those with a survival time of more than 12 weeks [17].

CONCLUSIONS

Low levels of vitamin D have been implicated in various conditions of chronic pain. Research has shown that vitamin D exerts anatomical, hormonal, neurological and immunological influences on the manifestation of pain, thus playing a role in the pathogenesis and maintenance of chronic pain states and associated comorbidity.

It is hypothesised that Vitamin D provides clinical benefits in patients with chronic pain. There are several observational studies that have shown that vitamin D supplementation provides some pain relief. Nevertheless, the results of some studies have often provided discordant outcomes. There are many reasons for these discrepancies. One point is the precise definition of serum $25(OH)D_3$ levels to determine its deficiency, normal range, and cut-off for toxicity. The difficulty in establishing pathophysiological levels of $25(OH)D_3$ deficiency has been attributed to variations in method (statistical tools), the difference in experimental assays used (technical), geographic latitude, or other variations in the individuals being studied. Hence, it has been argued that the purported "normal" range for serum $25(OH)D_3$ levels should be defined on an individual basis and within the clinical context. Serum variations may also result from genetic polymorphisms in vitamin D processing enzymes and changes in vitamin D pharmacokinetics and pharmacodynamics. Another level of complexity may arise from specific variations in the disease state of individuals, and this is particularly important in chronic pain, which shows extreme heterogeneity among individuals whilst perception of pain may be highly individualised. This latter point brings great challenges in the accurate assessment of pain especially when relying on self-reporting by the afflicted. Therefore, the development of reliable pain biomarkers that can be accurately applied to pain assessment in clinical trials is urgently needed. Hence, there is a need for large randomised controlled clinical trials that can take into account the many variables involved, in order to conclusively determine the analgesic benefit of vitamin D in chronic pain and whether or not the effect is limited to patients who are vitamin D deficient.

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