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EDITORIAL

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Dear Readers,
In this issue, two topics are discussed in depth, as usual by expert Authors who are working on them.
The first topic concerns an update on the possible role of vitamin D in atopic dermatitis. It is known that the skin is a central organ for vitamin D metabolism, representing both the site of its synthesis and a target organ. Vitamin D regulates both the proliferation and differentiation of keratinocytes and it is also involved in regulating the synthesis of ceramides, which are a key component of the corneocyte lipid envelope, thus helping to protect the skin from pathogenic chemical, physical and microbiological agents. Vitamin D also performs several actions on the skin's immune system, including induction of antimicrobial peptide synthesis, inhibition of antigen presentation by Langerhans cells and induction of regulatory T lymphocytes. So, patients with atopic dermatitis show genetic and acquired alterations in the formation and regulation of their skin barrier and a dysregulation in their immune response. Hence the possible role of vitamin D deficiency in the pathogenesis of certain inflammatory and immune-mediated skin diseases such as atopic dermatitis and the opportunity to exclude or treat it in affected patients.
The second topic addressed in this issue concerns recent epidemiological and clinical evidence indicating that some benefits of vitamin D supplementation, whether skeletal or extra-skeletal, may be limited to the daily dosage. Recent studies, including those from our School [1], have in fact shown pharmacokinetic and pharmacodynamic characteristics that justify the preferential choice of a daily supplementation strategy over that of boluses. Indeed, we showed that a daily dose, often considered less functional, is more effective than boluses (with the same cumulative dose) in restoring and increasing normal 25(OH)D levels. The explanation for this phenomenon must be sought in vitamin D's different anabolism-catabolism in relation to its supplementation schedule. Vitamin D boluses rapidly saturate 25-hydroxylase, which is responsible for the conversion of vitamin D_3 and D_2 to 25(OH)D, resulting in the induction of 24-25-hydroxylase, the enzyme responsible for the catabolism of vitamin D to 24-25(OH)D (the inactivated form). In other words, 25-hydroxylase saturation would limit the conversion of cholecalciferol boluses to the semi-active form, resulting in fewer biological effects. The 25(OH) hydroxylase reminds me of an oven where bread is baked daily, which needs a daily supply of flour to maximise production but would not benefit from an intermittent supply of flour, even if in surplus.

However, there is another possible though intriguing motivation for dosing with a daily strategy: the potential extra-skeletal immunomodulatory effect of vitamin D would in fact appear to be attributable to the direct activity of the 25(OH)D precursor, that is, cholecalciferol or vitamin D_3 on immune cells [2]. Actually, after exposure to a foreign pathogen, T lymphocytes express the vitamin D receptor, which, in the presence of adequate levels of vitamin D_3, transduces a signal of lymphocyte proliferation and the activation of adaptive immunity. Therefore, this particular immunological effect seems to be mediated by the "inactive" vitamin D precursor and not by the forms that are biologically active on mineral and bone metabolism. Hence, this effect appears to be independent of 25(OH)D concentrations, but more closely linked to the availability of

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vitamin D_3 in the bloodstream. Consequently, daily doses could have the distinct advantage of maintaining stably high levels of vitamin D in the circulation, whose very short serum half-life, on the order of a single day, is well known. On the other hand, it is also known that many, if not all, cells have the hydroxylase activity required for intracellular activation of vitamin D.

Do you want to bet that we are on the verge of discovering, as recently hypothesised [3], that the serum concentration of cholecalciferol is actually better than that of 25(OH)D in expressing an adequate vitamin D level? Happy reading!

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The role of vitamin D in atopic dermatitis

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Abstract

The skin is a central organ for vitamin D metabolism, representing both the site of its synthesis and a target organ. Vitamin D regulates both proliferation and differentiation of keratinocytes. Vitamin D is also involved in regulating the synthesis of ceramides, a key component of the corneocyte lipid envelope, which acts as an epidermal barrier, protecting the skin from chemical, physical and microbiological agents. Vitamin D also carries out several actions on the skin's immune system. Among these there is the induction of the synthesis of antimicrobial peptides such as hCAP18/LL-37 and β-defensin and it inhibits antigen presentation by Langerhans cells, whilst inducing the formation of regulatory T lymphocytes. Atopic dermatitis (AD) is the most common inflammatory skin disease, affecting up to 20% of the paediatric population and 5% of the adult population. Several epidemiological studies have shown an inverse correlation between AD prevalence and latitude, reduced exposure to sunlight and hypovitaminosis D. Most observational studies and meta-analyses have shown that vitamin D levels are lower in adults and children with AD than in controls. Vitamin D supplementation, either oral or secondary to exposure to UV radiation, is generally associated with an improvement in AD. Serum vitamin D dosing is recommended for patients affected by AD.

PHYSIOLOGICAL FEATURES OF VITAMIN D IN NORMAL SKIN

Vitamin D is a secosteroid known primarily for the regulation of the metabolism of calcium and phosphorus and the maintenance of normal skeletal architecture. The skin is a central organ for vitamin D metabolism, representing both the site of its synthesis and a target organ. Vitamin D can be taken through food or through supplementation in the form of vitamin D_2 (ergocalciferol) or D_3 (cholecalciferol) and is synthesised in the skin. The vitamin D precursor, 7-dehydrocholesterol (pro-vitamin D) is contained in the membranes of keratinocytes of the basal and spinous layers. The action of UVB radiation (290-315 nm) opens the B-ring of 7-dehydrocholesterol to generate pre-vitamin D_3 or cholecalciferol [1]. In temperate zones, UVB radiation may be insufficient for adequate vitamin D synthesis, especially during winter. Other factors that may inhibit cutaneous vitamin D synthesis include advanced age, dark phototypes, limited exposure of skin surface area and/or the use of sunscreens [2]. To become metabolically active, vitamin D undergoes two hydroxylation reactions in the liver and kidneys by enzymes of the cytochrome P450 family, generating 25-hydroxyvitamin D [25(OH)D], which is the main serum index of vitamin D repletion, and 1,25-dihydroxyvitamin D [1,25(OH)2D], the active form of vitamin D. Keratinocytes themselves already contain all the enzymes necessary for vitamin D metabolism, namely CYP27A1 and CYP27B1.

Vitamin D's physiological effects are mediated by the nuclear vitamin D receptor (VDR), which, after activation, interacts with the retinoid X receptor to form heterodimeric complexes that bind specific regions in the promoter of target genes [1]. There is also a non-genomic mechanism of action, mediated by a membrane receptor, which results in the transduction of multiple signalling pathways, including the regulation of intracellular calcium levels and the activation of phospholipase C-γ1. Therefore, keratinocytes respond to vitamin D in both an autocrine and paracrine manner.

In vitro studies have shown that vitamin D has a dose-dependent effect on the proliferation and differentiation of keratinocytes. Low concentrations of vitamin D promote keratinocyte

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Conflict of interest
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proliferation, whilst high concentrations inhibit it and promote epidermal differentiation [3].
This anti-proliferative action arises from the repression of cyclin D and the induction of cell cycle inhibitors such as p21cip and p27kip. Vitamin D-mediated epidermal differentiation requires VDR binding with the joint participation of two specific coactivators: DRIP and SRC. Keratinocyte differentiation is promoted through the increased synthesis of the K1 and K10 keratins and other proteins involved in barrier function, including filaggrin, involucrin, loricrin and transglutaminase [4]. Vitamin D is also involved in regulating the synthesis of very-long-chain glucosylceramides and their transport into the lamellar bodies. These lipids form a component of the corneocyte lipid envelope with an important barrier function [5]. Vitamin D also performs several actions on the skin's immune system. One of the most important of these is induction of the synthesis of antimicrobial peptides, such as hCAP18/LL-37 and β-defensin in keratinocytes and sebocytes, either through direct transcriptional induction or indirectly through the regulation of KLK5 and KLK7 serine proteases. Antimicrobial peptides alter bacterial membranes and virus envelopes and stimulate the innate immune response [6]. Vitamin D and its analogue, calcipotriol, exert an immunosuppressive effect on the skin by inhibiting antigen presentation by Langerhans cells and inducing regulatory T lymphocytes [7]. A serum concentration of approximately 30 ng/mL of 25(OH)D is the estimated cut-off value for defining an adequate level of vitamin D. Vitamin D requirements range from 1,500 IU/day for healthy adults to 2,300 IU/day for the elderly. Vitamin D deficiency affects about half of all young patients in the winter and nearly the entire elderly population. Vitamin D3 supplementation is useful for the treatment and prevention of hypovitaminosis D. In cases of severe deficiency, cumulative doses of between 100,000 and 300,000 IU are given over a period of 1-4 weeks. In general, after adequate correction of the vitamin deficiency, a daily preventive dose of between 800 and 2,000 IU/day can be set, depending on age and exposure to sunlight. Many studies have confirmed that daily doses of up to 4,000 IU are safe and there are no reports of intoxication at this dosage [8].

ATOPIC DERMATITIS

Atopic dermatitis (AD) is the most common inflammatory skin disease, affecting up to 20% of the paediatric population and 5% of the adult population [9]. AD is a complex disease with a multifactorial aetiology. Patients with AD show genetic and acquired alterations in the formation and regulation of the skin barrier and dysregulation of the immune response [9]. Among the abnormalities in the barrier function of keratinocytes, there is also a filaggrin deficiency, increased serine protease enzyme activity and reduced levels of total lipids and ceramide fractions of their cell membranes [10]. The pathogenesis of AD is dominated by an immunological imbalance of Th2 and Th22, and an increased release of IL-4 and IL-13, which are also involved in the regulation of IgE synthesis. IL-4, and to a lesser extent IL-13, stimulate the switch to IgE production by B lymphocytes and also reduce the production of ceramides, loricrin, involucrin, desmoglein 3 and filaggrin. An increased Th2 type inflammatory response also leads to a reduced production of antimicrobial peptides. Th1 and Th17 type responses modulate the development and progression of the disease in chronic phases [9]. The distinctive features of AD are eczematous lesions, intense itching, and a chronic, relapsing progression with periodic exacerbations. Acute AD lesions are erythematous and vesicular and become chronically reddened, scaly and lichenified. Lesion topography characteristically changes with age [9]. At onset, AD in infants may present as cradle cap of the scalp, which then spreads to the extensor surfaces of the extremities and face with exudative lesions, which characteristically spare the mid-facial region. In children and adolescents there is a typical localization in the folds (flexural eczema), commonly associated with involvement of the face, neck and upper part of the trunk (Fig. 1). In adults, AD most often manifests as chronic eczema of the hands or face with characteristic involvement of the eyelids and neck (Fig. 2) [9].
The objective of treating AD is to achieve and maintain clinical remission and to prevent relapses. Treatment comprises a remission induction phase and a maintenance phase. For mild to moderate forms, topical anti-inflammatory therapy with corticosteroids or topical calcineurin inhibitors, tacrolimus and pimecrolimus, may be sufficient and can be used with a "proactive" maintenance treatment schedule, usually twice a week. Systemic treatments are indicated for more severe and widespread forms or when there is involvement of sensitive or visible areas (face), for forms that are made more severe by significant itching or that cause a major impact on the quality of sleep or the quality of life. Systemic drugs currently available include systemic corticosteroids, cyclosporine and dupilumab, a fully human monoclonal antibody directed against the IL-4 and IL-13 α receptors. Dupilumab is the first biologic approved for the treatment of AD. It has an excellent efficacy and safety profile, and is indicated in cases of intolerance, ineffectiveness and/or contraindication to cyclosporine. In especially severe and treatment-resistant cases, azathioprine, methotrexate and mycophenolate mofetil may also be used. Phototherapy may be useful in treating moderate forms. In patients over 12 years of age, broadband UV (UVA + UVB = 290-400 nm), narrowband UVB (311-313 nm) and UVA1 (340-400 nm) can be used with benefit. The use of emollients as an integral part of AD therapy is strongly recommended by all major international guidelines. However, some

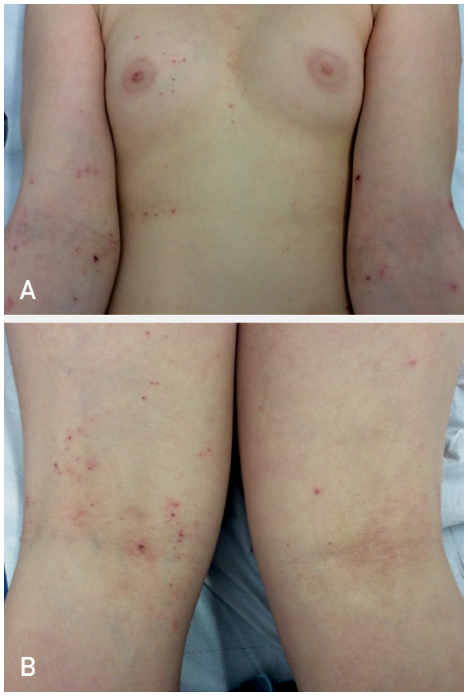

FIGURE 1.
Typical eczematous lesions on the chest and on the antecubital (A) and popliteal (B) fossa in a adolescent female patient with atopic dermatitis.

FIGURE 2.
Typical eczematous lesions on the eyelids (A) and neck (B) in a young woman with atopic dermatitis.

patients are reluctant to adhere to this recommendation due to the discomfort caused by the greasy sensation that some products leave on the skin as well as their cost [11].

ROLE OF VITAMIN D IN ATOPIC DERMATITIS

Several epidemiological studies have shown an inverse correlation between the prevalence of AD and latitude, reduced exposure to sunlight and hypovitaminosis D. Bryemo et al. observed an improvement in AD in Norwegian children who were moved to a sub-tropical country for 4 weeks [12]. Most observational studies have shown that 25(OH)D levels were lower in AD patients (both adults and children) than in the controls. For example, a Korean study involving more than 15,000 adults observed significantly lower vitamin D levels in AD patients compared with healthy controls [13]. A meta-analysis of eleven studies described a mean difference of 14 nmol/L (95% CI 25-2) between AD patients and healthy controls and 16 nmol/L (95% CI 31-1) in the paediatric population [14]. Other studies have described the association between hypovitaminosis D and increased disease severity, elevated IgE levels, allergic sensitisation and risk of food allergy, although the results do not overlap completely [15].
In particular, some studies have described this association only in children with AD but not in adults, whilst others have confirmed that there is an association between hypovitaminosis D and disease severity, but only in the presence of allergic sensitisation. On the other hand, Quirk et al. reported significantly higher vitamin D levels in children and adolescents with AD compared with controls [15]. It is likely that some methodological limitations in the studies may have influenced the variability of these results, including the failure to assess exposure to sunlight, vitamin D supplementation, and the fact that a single severity score assessment will not reflect long-term disease severity. Genetic polymorphisms in the VDR gene and in the enzymes involved in vitamin D metabolism could account for the variability of the observations [15].
Specifically, Heine et al. estimated the frequency of VDR gene polymorphisms in patients with severe AD, highlighting the increased prevalence of four specific haplotypes, which could affect disease severity by regulating the skin barrier and the local immune response [16]. Weber et al. reported that vitamin D deficiency is associated with superinfection by the more virulent strains of S. aureus and that vitamin D supplementation reduces colonisation by this bacterium, which is responsible for re-exacerbations of the disease [6].
Whilst topical application of vitamin D or its analogues may have an irritative effect on eczematous lesions, most studies indicate that oral vitamin D supplementation, at doses between 1,600 and 2,000 IU/day, have been associated with an improvement in AD, as measured by SCORAD and EASI scores [15].
Several hypotheses have been formulated to explain the beneficial effects of vitamin D supplementation on AD. These include normalisation of IL-2, IL-4, IL-6 and IFN-γ levels, an inhibitory effect on allergic responses with suppression of IgE production, normalisation of the barrier defect and increased production of antimicrobial peptides such as LL-37 [1]. In a double-blind randomised controlled trial in Mongolia with 104 children with AD, vitamin D supplementation (1,000 IU/day) was associated with improvement in AD as measured by EASI and IGA scores after one month [17]. Similar results have been shown in other randomised controlled trials. However, a Swedish prospective cohort study observed an increased risk of developing AD at six years of age in children who received a high dietary intake at between 5 and 10 months [18]. Finally, numerous studies have been conducted to

determine whether there is an association between maternal vitamin D levels and risk of AD in the unborn child, but with conflicting results [15].

CONCLUSIONS

Vitamin D may play an important role in the homoeostasis of healthy skin and in the pathogenesis of certain inflammatory and immune-mediated skin diseases such as AD. Hypovitaminosis D is an emerging risk factor for AD and is associated with well-known extra-cutaneous consequences on mineral metabolism and bone homoeostasis. Therefore, the serum dosage of 25(OH)D in AD patients is recommended, especially in winter, when its levels are expected to be lower especially in those patients who have been taking systemic and/or topical corticosteroids for a long time. In case of hypovitaminosis D, supplementation with vitamin D3 is recommended.

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Vitamin D supplementation: better daily or by bolus?

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Vitamin D is a pre-hormone and a dietary nutrient required for the normal function of specific physiological processes. Adequate levels of vitamin D are essential for the proper regulation of calcium-phosphorus homoeostasis and maintenance of the musculoskeletal system [1]. Recent findings have also highlighted some "extra-skeletal" properties of vitamin D [1,2]. Among these an important regulatory activity in the immune system has emerged [2].

Humans are able to synthesise vitamin D_3 through photochemical conversion. Ultraviolet B radiation leads to the conversion of 7-dehydrocholesterol to cholecalciferol by the skin. Several factors limit this process. These include the thickness of the stratum corneum (MORE often with advancing age), the angle of the earth's axis (which limits the amount of UVB useful for the production of vitamin D), and other environmental factors such as air pollution, cloudiness, etc [3,4]. Alternatively, vitamin D, in the form of vitamin D_3 (cholecalciferol), of animal origin and vitamin D_2 of plant origin (ergocalciferol), can be obtained from the diet or dietary supplements [5,6]. This source of vitamin D is essential when exposure to sunlight or the skin's response to ultraviolet radiation is insufficient, as in the elderly. Vitamin D, whether as D_3 or D_2, requires a two-step activation process to become biologically active. Vitamin D is transported in the bloodstream bound to a specific plasma protein: vitamin D binding protein (VDBP). Afterwards, within a few hours of synthesis or dietary absorption, vitamin D is hydroxylated in the liver, forming 25(OH)D (calcifediol). The next step is further hydroxylation largely, but not exclusively, by the kidney, forming $1,25(OH)_2D$ (calcitriol), the biologically active form of vitamin D [1]. To date, serum levels of 25(OH)D are the best indicator for assessing vitamin D status. It is now widely recognised that low levels of vitamin D (< 20 ng/mL) have detrimental effects on skeletal and extra-skeletal health [1].

In fact, among the various national and international scientific societies there is broad consensus on this threshold in the definition of vitamin D insufficiency [7]. Many epidemiological studies have shown that vitamin D deficiency is extremely widespread at all latitudes, especially among the elderly [8]. Quite a few observational studies have linked low serum vitamin D levels to the development or exacerbation of many chronic diseases. However, interventional studies on extra-skeletal health are still inconclusive, even though they have often been influenced by methodological problems [1,9]. Furthermore, there is still no consensus on the best supplementation scheme (dose, treatment frequency and duration).

Actually, in clinical practice, a wide variety of supplementation schemes have been proposed, often guided solely by the physician's preference. Supplementation schemes ranging from a few drops per day to mega-doses of vitamin D given over time, in some cases every six months, are used. The lack of uniformity of these regimens can be explained, at least in part, by the paucity of comparative pharmacokinetic data for the different treatment regimens. However, it has recently emerged that a daily dose, often considered less effective, is instead MORE efficient than boluses (at the same cumulative dose) in restoring normal 25(OH)D levels or increasing them (Fig. 1) [10]. Although this last study had no pre-determined clinical objective and was conducted on healthy subjects, who were followed for just a short time, it did provide valuable information on the pharmacokinetics of vitamin D. The explanation for this phenomenon should be sought in the different anabolism-catabolism of vitamin D in relation to any supplementation scheme. Vitamin D boluses rapidly saturate the hepatic 25-hydroxylase, which is responsible for the conversion of vitamin D_3 and D_2 into 25(OH)D, with the resulting induction of the 24-25-hydroxylase, the enzyme responsible for the catabolism of vitamin D to 24-25(OH) D (inactivated form) [11]. In other words, 25-hydroxylase saturation would limit the conversion of cholecalciferol boluses

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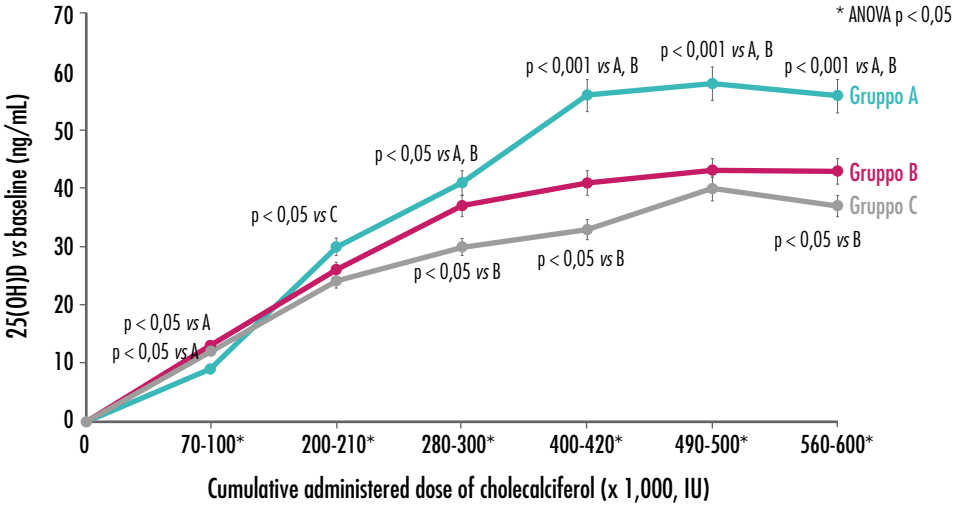

FIGURE 1.
Pharmacokinetics from different treatment regimens in vitamin D deficient patients. Blue line 10,000 IU daily, orange line 50,000 IU weekly, grey line 100,000 IU biweekly(from Fassio et al., 2020, mod.) [10].

to the semi-active form, resulting in fewer biological effects.

This hypothesis is supported by long-term clinical studies which suggest that the treatment schedule itself (i.e., bolus vs fractionated administration) may have a different impact on the efficacy of the treatment and the clinical outcome studied. For example, a recent meta-analysis of more than 40,000 individuals published in the prestigious JAMA Network Open journal showed that only daily doses of vitamin D and not intermittent doses alone were able to reduce the risk of fragility fracture. Specifically, not particularly high doses (400-800 IU daily) reduced the risk of hip fracture by 16% (RR, 0.84; 95% IC, 0.72-0.97) [12].

The evidence supporting the improved efficacy of the daily regimen in restoring normal 25(OH)D levels is therefore growing and increasingly convincing. In addition, it is interesting to note that several studies have shown daily administration schemes to be more promising in terms of both skeletal and extra-skeletal effects. A meta-analysis of randomised clinical trials of more than 11,000 patients published in 2017 showed that vitamin D supplementation is in fact able to significantly reduce the risk of acute respiratory infections (aOR, 0.88; 95% CI 0.81-0.96). The effect was particularly evident in patients taking daily or weekly doses (aOR, 0.81; 95% CI 0.72-0.91), whilst it was not apparent in patients treated with vitamin D boluses (aOR, 0.97; 95% CI 0.86-1.10) [13]. In addition, the protective effect of vitamin D supplementation was, as foreseeable, particularly strong in vitamin D-deficient patients (aOR, 0.30; 95% 0.17-0.53 in patients with pre-study 25(OH)D <10 ng/mL) but, surprisingly, patients with levels ≥ 10 ng/mL also had a tangible benefit from vitamin D supplementation (aOR, 0.75; 95% IC 0.60-0.95 in patients with pre-study 25(OH)D ≥10 ng/mL) [13]. In practical terms, daily vitamin D supplementation in patients with very low vitamin D levels (<10 ng/mL) is able to prevent 70% of infections. This translates into an NNT (number of patients you need to treat to prevent an event) of just 4 individuals. This shows an extraordinarily high efficacy considering that the NNT of the influenza vaccination is between 10 and 50 individuals [14]. Furthermore, the discussion on the efficacy of vitamin D in preventing and treating SARS-CoV-2 infection is also highly topical. To date there are robust epidemiological findings available showing here are robust epidemiological findings available showing that vitamin D deficiency is an important risk factor for contracting SARS-CoV-2 and for developing complications related to COVID-19 [15]. Indeed, it has been noted that over 70% of patients with COVID-19 have insufficient vitamin D levels 16 and that patients with severe respiratory failure have LOWER 25(OH)D levels than patients with non-severe COVID-19 [16]. Nevertheless, there is still little evidence to support the efficacy of vitamin D supplementation in preventing or treating COVID-19. Particularly, randomised clinical trials of daily vitamin D supplementation strategies have not yet been published.

The potential extra-skeletal immunomodulatory effect of vitamin D could be due to direct activity of the 25(OH)D precursors, cholecalciferol and ergocalciferol, on im-

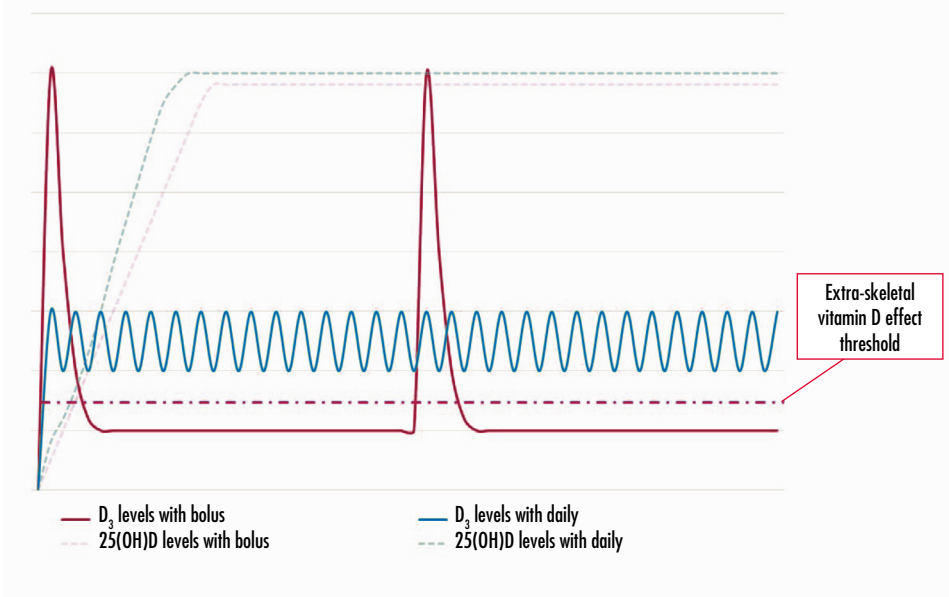

FIGURE 2.
Graph showing the extra-skeletal vitamin D threshold-effect hypothesis and the effects of bolus and daily administration on vitamin D and 25(OH)D levels.

mune cells [2]. After exposure to a foreign pathogen, T-lymphocytes express the vitamin D receptor, which, in the presence of adequate levels of vitamin D_3 or D_2, transduces a signal for lymphocyte proliferation and activation of adaptive immunity.
This particular immunological effect, which has been widely documented in vitro, is mediated by 'inactive' vitamin D precursors and not by the forms biologically active on mineral and bone metabolism. Therefore, this effect appears to be independent of 25(OH)D concentrations, but MORE closely linked to the availability of vitamin D_3 and D_2 in the bloodstream. Daily doses could therefore have the distinct advantage of maintaining stably high levels of vitamin D in the circulation by constantly stimulating immune T cells. On the other hand, bolus administrations are rapidly converted to 25(OH)D with circulating D_2 and D_3 levels dropping rather quickly [17]. Figure 2 shows the hypothesized different effect on extra-skeletal effects of vitamin D bolus compared to daily administration. In conclusion, we believe that there is now pharmacokinetic, pharmacodynamic and clinical evidence to justify the preferential choice of the daily supplementation strategy over the bolus strategy.

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