

# VITAMIN D

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Editorial

Immunomodulatory role  
of vitamin D  
in coeliac disease

Vitamin D  
and COVID-19:  
a glimmer of hope  
in the storm?

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# EDITORIAL

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**VITAMIN D**  
UpDates

2020;3(4):120-121

Dear Readers,

Until now, we have sorely lacked an article in the field of gastroenterology concerning vitamin D. Now we have one in this issue. In particular, we look at the subject of celiac disease, thanks to the contributions of colleagues with expertise as gastroenterologists. As you will see, the connection between celiac disease and vitamin D has a double aspect: on the one hand, intestinal lesions can lead to poor absorption of vitamin D, with the well-known negative effects on bone health, while on the other hand vitamin D deficiency is associated with abnormal inflammatory response, which may promote the onset and continuation of enteropathy, at least potentially. Indeed, the authors report evidence from the literature which leads us to believe that vitamin D can play a role in the pathogenesis of celiac disease, both by means of a direct protective effect on the intestinal barrier and by modulating the immune response in order to stimulate tolerance mechanisms. In particular, in this field as well we find a rationale for protective action on the part of vitamin D with regard to a "cytokine cascade", an inflammatory response which at excessive levels can cause serious complications (because of so-called "friendly fire"), as has also been hypothesized during the COVID-19 pandemic [1]. In this context, we recently suggested [2] that the past use of aminobisphosphonates, which in the long term reduce the level of circulating T  $\gamma\delta$  lymphocytes and hence the cytokine cascade responsible for the reaction in the acute phase, may account for the observed reduction of pneumonia and pneumonia-related fatalities in patients treated with these drugs [3].

In addition, in light of the current second wave of the SARS-CoV-2 pandemic, we believe we owe our readers an update on scientific progress regarding the COVID-19 disease. As the multiple immunomodulating effects of vitamin D are known and recognized, we felt obliged to include a bibliographical selection on the subject in this issue. I believe we can share the prudent, well-founded conclusions of the authors to whom we have entrusted this task: in their view, available data lends credence to a connection between vitamin D deficiency and susceptibility to and severity of infection by SARS-CoV-2.

As part of the research conducted during the previous pandemic wave, we studied the prevalence of 25(OH)D deficiency in patients hospitalized for COVID-19, examining in particular connections between vitamin D status and the seriousness of the illness [4]. Of the 61 patients included in the study, 72.1% showed 25(OH)D deficiency (< 20 ng/mL), while 57.4% had 25(OH)D levels < 15 ng/mL. Vitamin D deficiency was associated with increased risk of arterial PO2 < 60 mmHg, a threefold increase in the risk of altered PCR values, and increased risk of suffering from dyspnea at the beginning of the illness. Nevertheless, it will be clear to you that because we are dealing with a retrospective observational study these results do not allow us to attribute a causal role to vitamin D deficiency, even if we take the known effects of phlogosis on serum 25(OH)D levels into account, especially when severe [5,6]. Only the results of clinical trials that involve vitamin D supplementation can give us sure answers on this score. In any case, I believe that in the meantime it is best to avoid the risk of vitamin D deficiency. What do you think?

I wish you all well.

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# Immunomodulatory role of vitamin D in coeliac disease

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## Abstract

Quite recently, a growing number of studies have shown the extra-skeletal effects of vitamin D, especially in maintaining immunological homeostasis and preserving the intestinal barrier. As a result, the hypothesis of the involvement of vitamin D in the pathogenesis of many immune-mediated conditions seems now plausible. Among these, coeliac disease is a chronic inflammatory condition targeting the small intestine, that is triggered and sustained by the ingestion of gluten contained in some cereals by genetically susceptible individuals. Coeliac disease is the world's most frequent noncommunicable illness, whose prevalence ranges between 0.5 and 1.0%. However, in spite of the high – if not absolute – reliability of diagnostic tests, its real prevalence is much lower (roughly 1%) due to the variability of clinical features, including pauci-symptomatic cases, that is coupled with the limited knowledge of this condition among general practitioners. This generates the so-called "iceberg" phenomenon, in which diagnosed cases represent only the upper visible fraction of the total. In any case, our current understanding strongly recommends evaluation of serum vitamin D levels in both young and adult patients with coeliac disease, given that both the enteropathy and the possible bacterial overgrowth of the small intestine can lead to malabsorption of vitamin D, with obvious consequences on bone health. In addition, recent studies have proven its immunomodulatory role on all cell populations involved in immune response, while protecting the intestinal barrier and regulating the enterokinesis. Vitamin D deficiency may, therefore, represents an environmental factor that, together with gluten and genetic predisposition, is necessary for triggering and maintaining intestinal lesions in this pathological condition.

## INTRODUCTION

Very recent studies have given rise to the intriguing idea of the role of vitamin D in regulating immune response and preserving the intestinal barrier, one which is no less important than its known involvement in bone metabolism [1]. What is indeed emerging from research is the connection between vitamin D status and immune-mediated diseases, such as multiple sclerosis, type 1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus [2,3], to cite the best known. These illnesses constitute the true medical emergency in both western and developing countries: their prevalence is continuously on the rise and responsible for high direct costs, as well as for indirect ones linked to decreased quality of life and

to disabilities in a significant percentage of the population, above all working age adults [4]. They include chronic inflammatory intestinal diseases triggered by the complex interaction between genetic, immunological and environmental factors, a circumstance which accounts for their clinical variability [5]. Coeliac disease (CeD) is the most frequent enteropathy in the world, given that its prevalence reaches between 0.5 and 1.0% [6], with a large proportion of cases remaining undiagnosed [7]. Improved diagnostic capabilities combined with the pressure of environmental factors are contributing to a significant increase in its incidence [8]. Indeed a dual connection exists between CeD and vitamin D: on the one hand, intestinal lesions

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**Conflict of interest**  
Rachele Ciccocioppo and Luca Frulloni declare that they have no conflicts of interest.

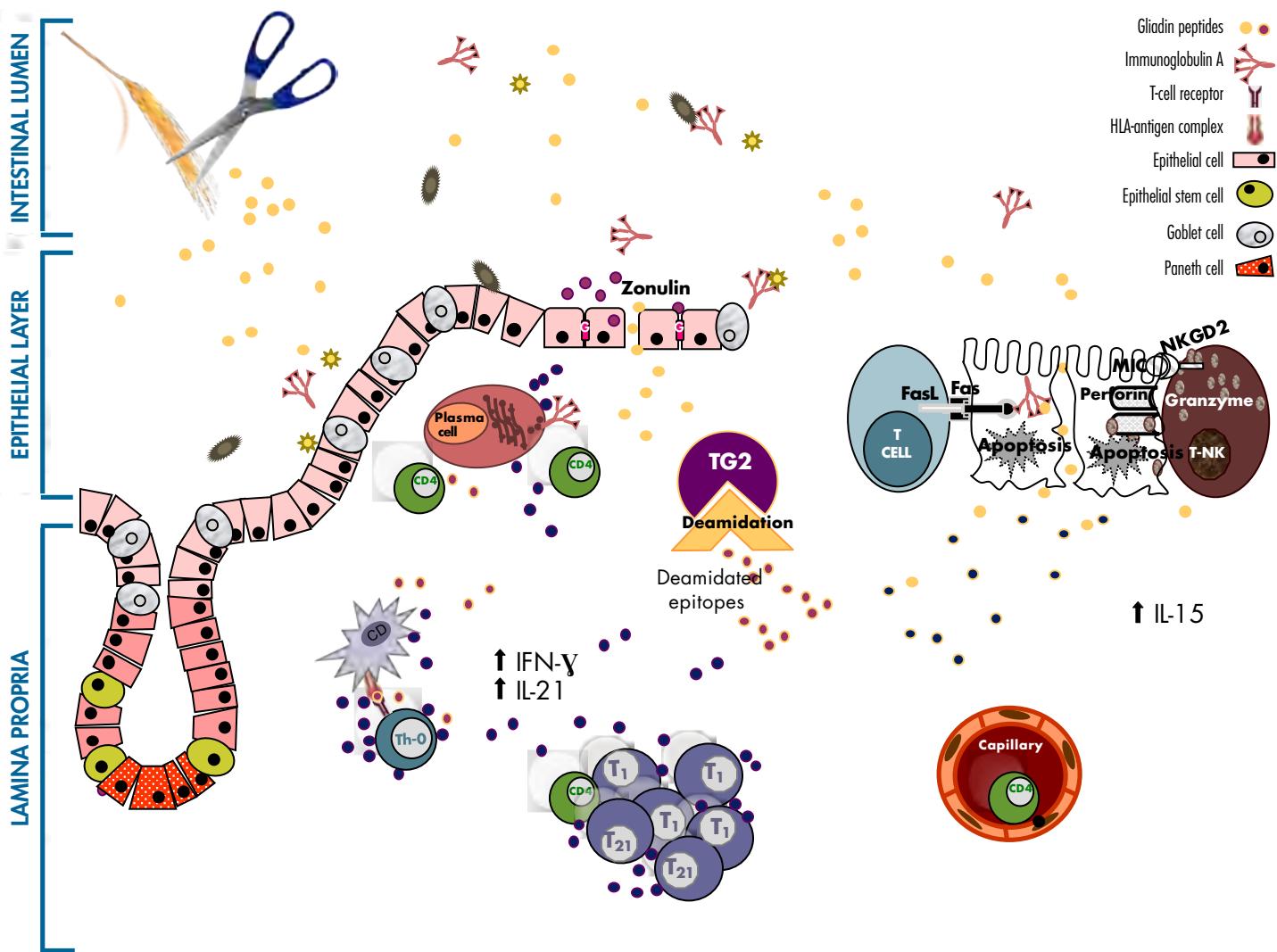
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DC: dendritic cell; Th-0: T helper-0; IFN: interferon; IL: interleukin; TG2: tissue transglutaminase type 2; G: inter-enterocyte junctional complex; NK: natural killer; FasL: Fas ligand; Fas: death receptor; NKG2D: natural killer group 2-member D; MHC: Major histocompatibility complex class-I-related chain molecules; Th21: T-helper 21; Th1: T-helper 1; T-NK: lymphocyte T natural killer.

**FIGURE 1.**

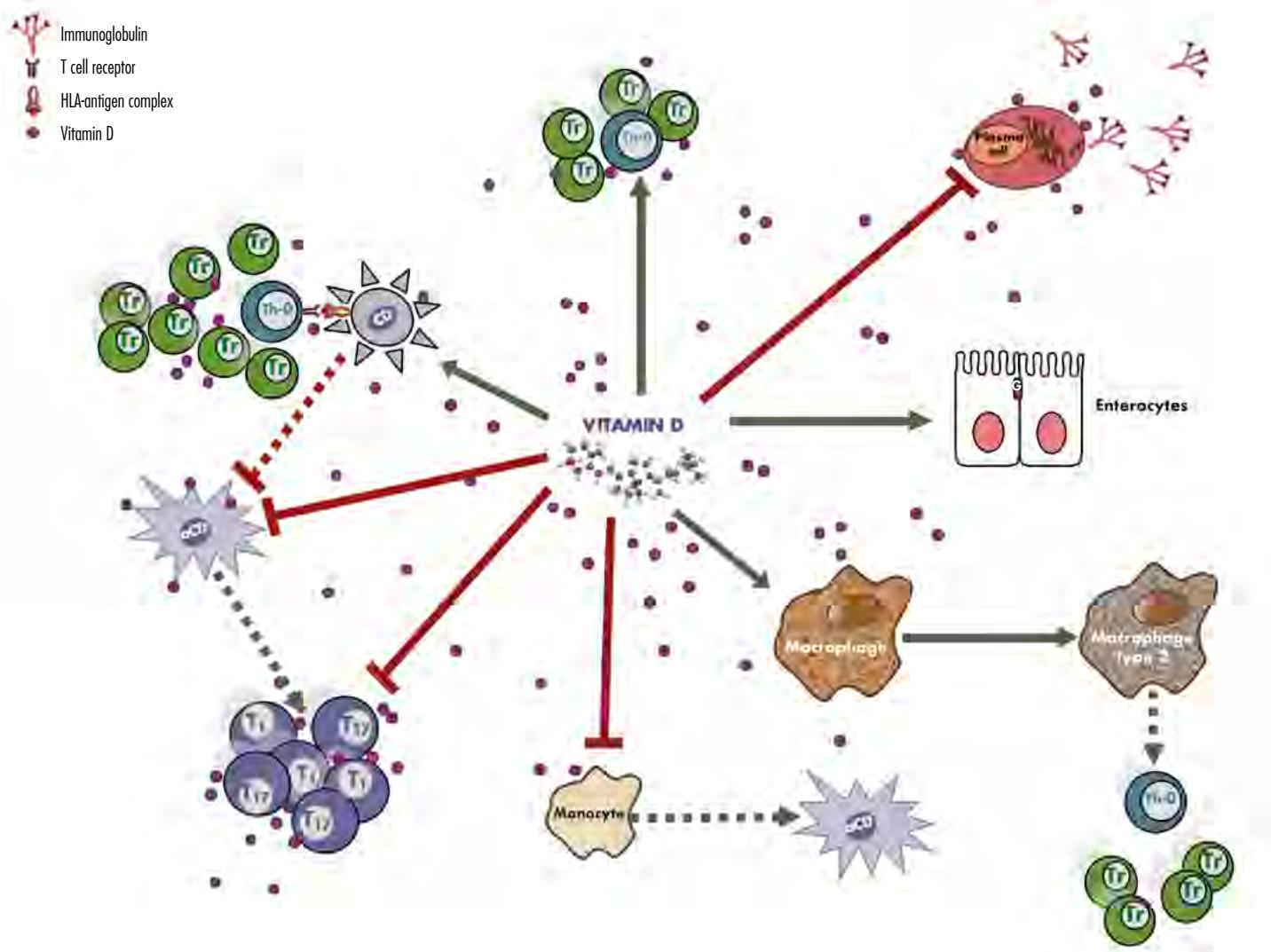
Immunopathogenesis and intestinal lesions of coeliac disease. See text.

can lead to poor absorption of vitamin D, with negative effects on bone health [9], while on the other hand vitamin D deficiency is associated with abnormal inflammatory response [10], which may promote the onset and persistence of enteropathy, at least potentially. This mini-review begins with the immunopathogenesis of CeD (Fig. 1). We then look at the evidence that has so far been collected on the effects of vitamin D (and of its deficiency) on innate and adaptive immunity and on the intestinal barrier (Fig. 2). Finally, we review current available data on CeD.

### COELIAC DISEASE

This pathology can develop in genetically predisposed subjects upon ingesting gluten; indeed eliminating gluten from their diets is the only therapy available today [11]. It is an autoimmune disease that especially targets the small intestine, although it can affect the skin (dermatitis-herpetiformis) or the cerebellum (gluten ataxia) in a certain number of cases [12]. Pathogenetic mechanisms connected with the disease cause localized lesions in the intestinal mucous membrane and are characterized by an increase in intraepithelial lymphocytes, crypt hyperplasia,

various degrees of villi atrophy and polymorphic inflammatory infiltrate of the lamina propria [13]. In this regard, CeD represents a condition that is well understood, in that the haplotypes for genetic susceptibility (HLA-DQ2/8), the external trigger (gluten) and the autoantigen (the tissue transglutaminase enzyme) are all known [11]. Nonetheless, genetic predisposition concerns over 30% of the population, and gluten is a basic foodstuff in the diets of nearly all the world's peoples. It is therefore clear that other factors are involved which enable lesions to develop and persist; these other causes help



aDC: activated dendritic cell; DC: dendritic cell; G: inter-enterocyte junctional complex; Treg: regulatory T lymphocyte; Th-0: T helper 0; Th1: T helper 1; Th17: T helper 17.

## FIGURE 2.

Immunomodulatory and protective effects of vitamin D on intestinal epithelium. See text.

us explain the age variability in the onset of this disease. Indeed, researchers speak of missing environmental factors [14], which include the microbiota, the type of delivery and feeding, the age of weaning, viral infections, and – recently – vitamin D levels. Each of these elements is an object of current research.

### *Immunopathogenesis*

Gluten represents the protein component contained in some cereals, such as wheat, barley, rye and oats. It remains in flours after

the bran has been eliminated by grinding and the starch by centrifugation [15]. What is actually involved is a mixture of proteins, which are soluble in alcohol: gliadins in wheat, hordeins in barley, secalins in rye and avenins in oats. Their peculiarity consists in the fact that they are rich in proline and glutamine, which gives various types of flour the properties necessary for rising and breadmaking. The human intestine does not produce enzymes (prolyl-endopeptidase) able to break the bonds between these amino acids. As a result, following chemical

digestion performed by gastric acidity and enzymatic digestion by intestinal peptidases, oligopeptides remain which cannot be broken down further [16]. When the intestinal barrier is impaired as a result, for example, of a viral infection or dysbiosis [17], oligopeptides pass through the epithelium and reach the lamina precisely where the immunocompetent cells are located, whose function is to maintain immunological tolerance with respect to the myriad of bacterial and dietetic antigens present in the intestinal lumen. Furthermore, a direct mechanism

causing damage to the barrier has also been hypothesized, which therefore may lead to increased intestinal permeability by means of the release of zonulin [18]: this occurs following the bonding of oligopeptides with the chemokine CXCR3 receptor expressed on the enterocytes, which in turn causes the molecules which form the tight junctions to disassemble [19]. Together with the adherens junctions and the basal complex, these junctions bind the enterocytes together so as to guarantee an extremely selective passage of the molecules, via trans- or paracellular transport, thus contributing to the integrity of the anatomo-functional unit called the intestinal barrier [20].

Other elements that make up the barrier include secretory immunoglobulin A (SIgA), the mucus layer that lines the enterocytes, the intraepithelial lymphocytes, as well as all other cell populations present in the lamina itself, which form the so-called gut-associated lymphoid tissue, on which depends immunological homeostasis [21]. Indeed, the state of antigen cells present in the lamina – whether dormant or active – determines the fate of the immunological response: tolerogenic or inflammatory [22]. Recent studies have indeed shown that the presence of pathobiont species in the intestinal microbiota, together with gliadin peptides, causes the activation of the dendritic cells, which in turn perceive the oligopeptides as antigens: they group them with HLA-DQ2/8 molecules and present them to the T CD4+ lymphocytes, triggering an inflammatory storm rather than a tolerogenic response [23]. The presence of an inflammatory micro-environment also results in the activation of the enzyme tissue transglutaminase. On the one hand, this enzyme represents the autoantigen of CeD [24], while on the other it makes a selective deamidation of such oligopeptides, in particular of 33-mer, substituting glutamine residues with glutamic acid. This deamidation makes oligopeptides immuno-dominant and therefore able to amplify the proliferative and secretory response of T-specific lymphocytes [25]. Thus stimulated, the latter produce a pro-inflammatory cytokine cascade, largely dominated by interferon  $\gamma$  and interleukin-15, with the subsequent activation of CD8+ cytotoxic lymphocytes, macrophages, and the natural killer cells which are ultimately responsible for villi atrophy [26].

Villi atrophy is the result of exaggerated enterocyte apoptosis due both to the cytolytic

action mechanisms of Fas/LigandFas and of perforin-granzyme and to the detachment of the basal membrane, which is not balanced by increased proliferation at the crypt level [27]. This compromised condition contributes to the loss of the intestinal barrier function because the mucosa surface is now lined with immature cells that form an inefficient junction complex. Finally, T-helper lymphocytes have epitopes to B lymphocytes which produce specific antibodies after differentiating in plasma cells [28]; this count, which can be measured in a patient's serum, has very high diagnostic accuracy [29].

## IMMUNOMODULATORY EFFECTS OF VITAMIN D

The vitamin D receptor (VDR), located at the level of the nucleus and responsible for the biological effects of vitamin D, is codified by a highly polymorphic gene that forms part of the superfamily of receptors for steroids [30]. Recently, the expression of this receptor has also been identified in tissues which are not involved in bone and mineral metabolism, and in particular in cells of the immune system, such as those presenting the antigen [31]. For this reason, some VDR polymorphisms may increase or decrease susceptibility to immune-mediated diseases, including CeD [32]. This fact has motivated a series of studies that have shown that vitamin D is involved in immune response, both innate and adaptive [33]. Indeed, the enzyme that converts 25-hydroxyvitamin D into its active form is also expressed in monocytes-macrophages and is activated following the bond of the toll-like receptors on their surfaces with the respective viral and bacterial antigens, with the result that defenses are strengthened against infections [34]. In addition, vitamin D inhibits the differentiation of monocytes in dendritic cells, thereby reducing the possible appearance of the antigen to trigger an inflammatory response [35].

As has been demonstrated in *in vitro* studies, vitamin D also acts directly on dendritic cells by inhibiting their maturation and therefore their antigen-presenting ability and by favoring the acquisition of a tolerogenic profile [36,37].

Regarding adaptive response, vitamin D reduces the differentiation of T lymphocytes towards a pro-inflammatory profile while promoting the expansion of their regulatory activity [38]. Furthermore, T lymphocytes that express high levels of VDR on their

surfaces are believed to be sensitive to an immunomodulatory action from vitamin D, acquiring an anti-inflammatory power. This fact is of great interest in the context of the pathogenesis of CeD, in that the gliadin-specific T lymphocytes are the agents mainly responsible for damage of the mucous and for the process that favors the onset of lymphoma connected to this type of chronic inflammation [39].

Concerning its effects on B lymphocytes, *in vitro* studies have also shown that vitamin D is able to reduce their differentiation in plasma cells and to increase their apoptosis by ultimately causing a reduction in the production of immunoglobulins and therefore of autoantibodies [40].

What is more, VDR is also located on the enterocytes, where it regulates their proliferation, differentiation and apoptosis. It effectively governs the enterokinetics, thereby playing a primary role in the defense mechanisms and functionality of the intestinal barrier [41]. Along these lines, we should mention the studies of Chen et al., which show that vitamin D has a protective effect on the epithelial barrier, both *in vitro* and *in vivo*, by signaling through a myosin light-chain kinase-dependent enzyme, which in turn is activated by the increase of the kB nuclear factor caused by an inflammatory stimulus [42,43]. In particular, the myosin light-chain kinase-dependent enzyme acts directly on the assembly on the actin filaments, causing a contraction of the cytoskeleton and therefore the destruction of the tight junctions. This phenomenon is of great importance if we consider that the disassembly of the latter has already been shown in CeD [44].

Later, the research group led by Dong confirmed the protective effect of vitamin D with regard to the tight junctions of the enterocytes. These studies employed an *in vitro* model of a single layer of CaCo<sub>2</sub> cells in which the rupture of the epithelial barrier was induced by gliadin peptides, as well as an *in vivo* gluten-sensitive model [45]. In both cases, vitamin D was able to inhibit the release of the zonulin induced by gliadin and to protect the integrity of the tight junctions, thereby maintaining the function of the barrier. Yet the first study to have brought attention to the possible extra skeletal role of vitamin D in children with CeD was that conducted by Tanpowpong et al. [46]. These authors showed how vitamin D deficiency could contribute to a compromised intestinal barrier, making subjects more susceptible to

enteric infections and consequently to the risk of developing abnormal immune responses vis-à-vis antigens present in the intestinal lumen. This finding has completely revolutionized the way in which we clinical doctors think about vitamin D in CeD, in a context in which deficiency had been considered a mere effect of enteropathy and not a possible cause. Finally, in a cohort of adults with CeD, vitamin D deficiency was correlated to increased frequency of psoriasis, though not to other autoimmune diseases [47].

Taken together, these findings lead us to believe that a simple connection of serum vitamin D level to CeD is reductive. For this reason, we hope that future studies will focus on further evaluating the overall effects of this disease so as to determine the risk of developing it, of prolonging organ damage, and perhaps of developing further complications.

## CONCLUSIONS

From the literature we have reviewed, it seems evident that vitamin D can play a significant role in the pathogenesis of CeD, both by means of a direct protective effect on the intestinal barrier and by modulating the immune response to promote tolerance mechanisms. These facts lead us to hypothesize that programs designed to prevent deficiency can contribute to limiting increased incidence of not only this pathology but also of many other chronic inflammatory diseases. In this light, it is worth remembering that vitamin D deficiency in women during pregnancy seems to be connected to an increased risk of developing autoimmune diseases, including CeD, above all during the first two years of life [48]. Supplementing the entire population is obviously not conceivable, in particular those who already have adequate daily vitamin D intake, given that excessive levels not only compromise bone health but also upset immunological homeostasis, with excess vitamin D stimulating Th2 polarization [49,50]. It is therefore essential to continue prevention and screening programs which are able to identify vitamin D deficiencies in the population in order to limit large-scale consequences.

## Acknowledgments

We would like to warmly thank those coeliac patients and their families for their willingness to participate in studies which aim to improve our knowledge about this disease and consequently about their condition.

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# Vitamin D and COVID-19: a glimmer of hope in the storm?

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VITAMIN D  
UpDAtes

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As with many recent pathologies, COVID-19 (CORONAVIRUS Disease-2019) has biological characteristics as well as clinical and medical imaging manifestations which are truly unique. In some subjects, COVID-19 is the result of infection by Severe Acute Respiratory Syndrome Corona-VIRUS-2 (SARS-CoV-2), the seventh type of coronavirus able to infect humans thus far [1]. The infection spread rapidly throughout the world, beginning from China [2]. The virus is highly transmissible, mainly by means of droplets emitted when speaking or breathing, or by direct contact (even if the virus is also detectable in feces in more advanced stages of the infection, which suggests the possibility of infection by an oral-fecal route) [1].

The incubation period of COVID-19 ranges from one day to two weeks, attaining a peak between the third and seventh day. Clinical expressions vary considerably, from total asymptomatic to severe conditions, such as Acute Respiratory Distress Syndrome (ARDS). The most common symptoms in mild to moderate forms are fever, asthenia and dry cough, which can be followed or accompanied by headache, nasal congestion, pharyngodynia, myalgia and arthralgia. In rare cases the gastro-intestinal system is involved (especially in children), with nausea, vomiting and diarrhea [1]. A variable proportion of infected subjects develops respiratory difficulties, hypoxia, desaturation and tachypnea, especially during the second week of illness. These are the typical signs of severe pulmonary infection, which can develop into bilateral interstitial pneumonia. The latter condition can in turn unfortunately evolve into ARDS, which is characterized by significant levels of morbidity and mortality [1]. The emergence of disorders in the coagulative system is also frequent, with thrombocytopenia potentially increasing the risk of hemorrhages, whether or not these are associated with other hematological conditions, such as peripheral thrombosis, deep vein thrombosis, pulmonary embolism and dis-

seminated intravascular coagulation (DIC) [1]. Given the current lack of antiviral treatments, clinic management of the illness is essentially based on controlling the abnormal inflammatory response and sustaining respiration in a hospital environment. These limitations explain why the pandemic has been able to upset even the most developed economic and health care systems, forcing authorities to develop new plans for the allocation of efforts and resources. It goes without saying that in such a moment of crisis attention is diverted from all situations that are not considered "essential". Yet are we absolutely certain that this is the correct strategy? An interesting editorial, which focuses in particular on the problem of osteoporosis, has been recently published on this question [3]. The authors quote a maxim of Jawaharlal Nehru (Gandhi's spiritual heir), who once said, "Every little thing counts in a crisis." These words are food for thought, especially as until now we have been in possession of limited data on truly effective treatments and on the factors able to condition our susceptibility to the infection and to determine its seriousness.

Undoubtedly, a vitamin is a "little thing", although vitamin D in particular (or its deficiency, to be precise) can play a significant role, including with regard to its much debated extra skeletal effects. Even though we are in full agreement with the European Society for Clinical and Osteoarthritis (ESCEO) that at the moment we do not possess sufficient evidence to recommend the use of vitamin D supplementation to prevent and/or treat extra skeletal pathologies [4], still we must also keep in mind that it was this very position paper that underlined the growing amount of available data (especially indirect, though also direct) which uphold the extra skeletal effects of vitamin D [4].

At present, infection from SARS-CoV-2 seems to occur more frequently and aggressively (in terms of mortality) in the countries of Southern Europe (Italy and Spain in particular), the

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#### **Conflict of interest**

Davide Gatti has received professional fees from: UCB, Celgene, Eli Lilly, MSD Italia, and Novartis.

Angelo Fassio has received professional fees from: Abiogen, Novartis, and Neopharmed.

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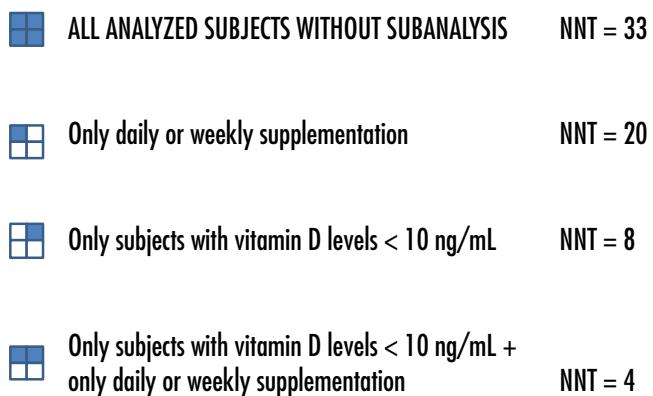
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**Number of patients who must be given vitamin D supplementation (NNT) to avoid acute respiratory infections (from a metanalysis of 25 studies with over 11,300 subjects)**



**FIGURE 1.**

Data from this wide-ranging metanalysis show that vitamin D supplementation is able to significantly reduce the risk of acute respiratory infections. Protection (in terms of NNT) is more evident in subjects with serious deficiency and appears particularly relevant when administration occurs daily or weekly (from da Martineau et al., 2017, with modifications) [6].

same ones whose populations show greater rates of hypovitaminosis D [5]. In Italy, the most affected regions are in the north, which have less sun exposure compared to those of the south, especially during winter. The elderly, and in particular those in long-stay care facilities, in whom hypovitaminosis D is effectively endemic, are those who have suffered the most dramatic consequences of this illness. The same holds true for obese subjects, who also have a high risk of vitamin D deficiency. As we have seen, COVID-19 is for the most part an infective respiratory disease, while the bulk of data supporting the claim that vitamin D has a significant effect in preventing and mitigating respiratory infections has become more substantial in recent years.

An interesting metanalysis published in 2019 analyzed data of over 11,300 patients from 25 randomized trials, showing a protective effect from vitamin D supplementation with regard to acute respiratory infections; this effect was not only statistically but also clinically significant, with a number needed to treat (NNT) of 33. Obviously, the effectiveness of supplementation was greater in subjects with severe deficiency (NNT = 8) (Fig. 1). Nonetheless, this protective ac-

tion on the part of vitamin D was not apparent in the sub-analysis of 15 studies which administered supplementation by means of bolus treatments, suggesting that the shielding effect was mainly achieved by daily or weekly supplements (10 studies), in which cases the findings are particularly interesting (Fig. 1) [6].

Another metanalysis focused on patients with COPD (chronic obstructive pulmonary disease), demonstrating once again that vitamin D supplementation is able to effectively reduce by half the number of moderate/severe respiratory exacerbations in patients with baseline deficiency (<10 ng/mL) [7]. This latter observation seems significant, given the great number of negative clinical studies with regard to vitamin D that have been published recently, which, unfortunately, all share the shortcoming of enlisting a majority of subjects whose vitamin D levels are adequate or even well above the ideal maximum [8]. Indeed, we must consider vitamin D a micronutrient rather than a pharmacological agent. As a result, supplementation is recommended and effective only in conditions of deficiency.

The immunomodulatory role of vitamin D has been known for some time. It is able to sup-

port innate immunity by the production of antimicrobial peptides, such as cathelicidins, defensines and IL-37. In addition, through the modulation of the main pro-inflammatory cytokine, including IL-6, TNF- $\alpha$ , and interferon gamma, it is able to act on adaptive immunity by controlling the response mediated by Th1 lymphocytes [9]. Clearly this control begins to falter at deficient levels of vitamin D, whose action is nonetheless restored following adequate supplementation. A recent study on cells of the respiratory epithelium has shown that pretreatment with physiological concentrations of vitamin D metabolites (calcifediol or calcitriol) can produce temporary resistance to the rhinovirus infection (Rv-16) and attenuate this virus's production of adhesion molecules required by both rhinovirus and *Streptococcus pneumoniae*. This process accompanies activation of the gene for cathelicidin and the modulation of NF- $\kappa$ B, which represent further possible mechanisms at the base of vitamin D's protective effects with regard to the rhinovirus infection and bacterial superinfection [10].

Concerning the SARS-CoV-2 virus, data are still limited at present, although a preliminary report of a study that assessed the antiviral potential of various molecules points to the inhibitory effect of calcitriol on nasal epithelia that have been infected by the virus [11]. This datum is of particular interest if we consider an Israeli study on 14,000 subjects tested for infection by SARS-CoV-2 who had previously received at least one serum dose of 25-hydroxyvitamin D [25(OH) D]. The findings show that suboptimal vitamin D levels (< 30 ng/mL) constitute a potential risk factor for infection by SARS-CoV-2, for contracting the COVID-19 illness as a result, and in particular for the need for hospitalization [12]. These results appear in line with those of an American study on a sample of 489 subjects whose vitamin D status had been measured the previous year. Of this cohort, 71 subjects tested positive for the SARS-CoV-2 infection. The study reported that the state of being "probably" deficient (circulating levels of 25(OH)D < 20 ng/mL, or 1,25(OH)2D < 18 pg/mL) was associated with a 1.77-time greater risk of testing positive [13].

On the other hand, a similar study conducted in the U.K. did not reproduce these results [14], even if the "anamnestic" values of vitamin D metabolites were perhaps not representative of real conditions at the time of infection; this circumstance constitutes a

limitation of this type of scientific research, one which is by no means insignificant. An interesting finding from the above mentioned American study [13] is that of the 48 subjects who were initially deficient and who then achieved adequate levels from vitamin D supplementation, the risk of testing positive for SARS-CoV-2 overlapped with the same risk in subjects who had sufficient levels from the start. This result indeed seems to support the claim for the protective effect of vitamin D supplementation when it is possible to normalize a subject's vitamin D status. The limited number of cases, however, results in a confidence level that is too wide to provide certain substantiation of this hypothesis. Meanwhile, a Swiss study used a different study design to evaluate a cohort of patients with suspected COVID-19 symptoms at an interval of several weeks following testing. Findings showed that vitamin D levels were significantly lower (median ca. 11 ng/mL) in subjects who tested positive compared to those testing negative [15]. This finding seems of interest, although we cannot exclude the possibility that the viral infection itself was responsible for the vitamin D deficiency. The relatively short duration of the infection (only a few weeks), however, makes this hypothesis less likely.

In addition to playing a potentially protective role vis-à-vis the infection, vitamin D may also affect the evolution of its severity, as is suggested by the data concerning hospitalizations in the above mentioned Israeli study [12]. The modulating/suppressing action of a possible excessive Th1 response on the part of vitamin D may in fact constitute a contributing factor in counteracting the cytokine storm at the base of lung damage and progression toward ARDS [9]. Indeed, vitamin D deficiency has been shown to be associated with greater risk of developing ARDS [16]. In addition, achieving adequate levels could aid in reducing the alveolar-capillary damage that occurs in deficient subjects [16]. This protective capacity of vitamin D seems to be secondary with respect to the local action of the active metabolite calcitriol on the renin-angiotensin system, which is generated by means of a direct effect on the expression of ACE enzymes [17]. This finding is of especial significance if we bear in mind that ACE-2 is believed to be the key receptor for SARS-CoV-2 infections. As is well known, the virus binds by means of a spike protein at the ACE-2 receptor, enabling it to

then penetrate pulmonary cells; it later acts by downregulating both the activity and expression of this enzyme [9].

In conclusion, available data in our view lends credence to a connection between vitamin D deficiency and susceptibility to and severity of infection by SARS-CoV-2. Following this reasoning, various interventional studies have been conducted on patients diagnosed with severe infections. This virus represents a challenge that many experts, ourselves included, believe will be difficult to overcome [18]. In these patients, in fact, the abnormal inflammatory response is probably too far developed in order to hypothesize a significant benefit from vitamin D supplementation, even in subjects with high levels of deficiency. In addition, the steroid drugs or immunosuppressants used in these cases produce effects which mask the potential action of vitamin D, which, after all, is a micronutrient. In any case, we believe that in light of the prevalence of vitamin D deficiency these patients should be given supplementation.

A more promising perspective is rather offered by research on the benefits of supplementation (daily or weekly) in reducing susceptibility to infection and progression toward more severe forms. In this light, we continue to fear the impact that AIFA's "nota 96" may have had on the prevalence of vitamin D deficiency, not only in subjects with compromised bone health but also in those most at risk of contracting COVID-19. We therefore wish to emphasize the need for forceful and prompt advocacy along these lines, before the onset of winter and a possible new wave of the pandemic, above all if we believe that "every little thing counts in a crisis."

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